29 July 2005

Science

Vol. 309 No. 5735
Pages 653–832 $10

Drug Discovery

BIG RISKS, BIG REWARDS

125 YEARS OF GLOBAL Science

AAAS

Sensitivity always wins out.

Achieve reliable PCR results from precious, diverse samples with the PicoMaxx™ PCR System.

The PicoMaxx™ High Fidelity PCR System* from Stratagene provides maximum PCR sensitivity and reliability. Get improved yields from low template amounts and achieve successful amplification from small, diverse samples that typically result in unreliable amplification. This economical, high fidelity enzyme blend provides great results up to 10 kb.

- **Low cost per unit**
- **High specificity hotstart**
- **Master mix format available**

Need More Information? Give Us A Call:

Stratagene USA and Canada
Order: (800) 424-5444 x3
Technical Services: (800) 894-1304

Stratagene Europe
Order: 00800-7000-7000
Technical Services: 00800-7400-7400

Stratagene Japan K.K.
Order: 03-5159-2060
Technical Services: 03-5159-2070

www.stratagene.com

Visit www.stratagene.com/amplification to learn more.

PicoMaxx™ High Fidelity PCR System	**100U**	**600420**
PicoMaxx™ High Fidelity PCR System	**500U**	**600422**
PicoMaxx™ High Fidelity PCR Master Mix	**100 rxn**	**600650**

* U.S. Patent Nos. 6,734,293, 6,489,150, 6,444,428, 6,379,553, 6,333,165, 6,183,997, 5,948,663, 5,866,395, 5,556,772, 5,545,552 and patents pending.

PicoMaxx™ is a trademark of Stratagene.

Purchase of this product is accompanied by a license under the foreign counterparts of U.S. Patents Nos. 4,683,202, 4,683,195 and 4,965,188 for use in the polymerase chain reaction (PCR) process, where such process is covered by patents, in conjunction with a thermal cycler whose use in the automated performance of the PCR process is covered by the up-front license fee, either by payment to Applied Biosystems or as purchased, i.e., an authorized thermal cycler.

STRATAGENE®

Launch Your Discovery with MISSION™ RNAi

Introducing the MISSION shRNA Library of The RNAi Consortium

The RNAi Consortium (TRC), based at the Broad Institute of MIT and Harvard, is developing pre-cloned MISSION shRNA libraries targeting the human and mouse genomes. As a member of TRC, Sigma-Aldrich is a principle collaborator and global supplier of the lentiviral based shRNA vector collections for use in transient or stable transfection, as well as viral particle generation. The complete libraries will target 15,000 human and 15,000 mouse genes.

You'll Discover:

- *~35,000 clones available targeting diverse gene families*
- *Renewable resource compared to siRNA*
- *Long term knockdown and phenotypic studies*
- *Lentiviral particles facilitate silencing in difficult cell lines*
- *Sequence-verified clones*

MISSION RNAi

Member of *The RNAi Consortium*

MISSION is a trademark belonging to Sigma-Aldrich Co. and its affiliate Sigma-Aldrich Biotechnology LP. The RNAi Consortium shRNA library is produced and distributed under license from the Massachusetts Institute of Technology.

*Experience the **MISSION shRNA** libraries for yourself.*
*Search our target gene database at: **sigma-aldrich.com/mission_px4***

Plus, register to receive our latest literature and updates on upcoming product formats and gene family sets.

sigma-aldrich.com

LEADERSHIP IN LIFE SCIENCE, HIGH TECHNOLOGY AND SERVICE
SIGMA-ALDRICH CORPORATION • BOX 14508 • ST. LOUIS • MISSOURI 63178 • USA

SIGMA®

GE Healthcare

Why do 100,000 scientists trust GE Healthcare for all their protein purification needs?

Here's Ä clue.

To 100,000 scientists worldwide, the name ÄKTA™ has always meant outstanding protein purification, and now it's brought to you by GE Healthcare. With the ability to purify virtually 100% of all biomolecules, the ÄKTAdesign™ platform can handle the toughest of challenges. Whatever the scale, from laboratory, to process development and manufacturing, there's an ÄKTAdesign system to meet every need. All systems in the ÄKTAdesign family work with the intelligent UNICORN™ software, which makes it easy to control every stage of your purification processes. Accurate, reproducible results just take a little pure imagination.

Visit www.amershambiosciences.com/aktadesign

imagination at work

GE15-05

Science

SPECIAL ISSUE

DRUG DISCOVERY: BIG RISKS, BIG REWARDS

Identifying new medicines and bringing them to market is a huge gamble—and the stakes are high. A special section examines the world of drug discovery and the scientists who work in it. [Illustration: Stephen R. Wagner]

Volume 309
29 July 2005
Number 5735

INTRODUCTION

NEWS

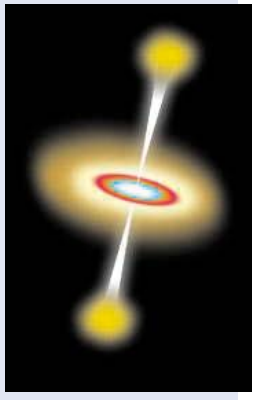
Related Editorial page 669

For related online content, see page 663 or go to www.sciencemag.org/sciext/drugdisc05/

DEPARTMENTS

688

NEWS OF THE WEEK



714 & 746

NEWS FOCUS

LETTERS

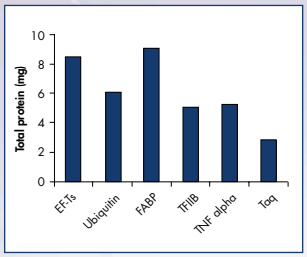
BOOKS *ET AL.*

POLICY FORUM

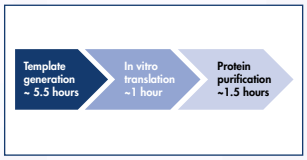
Contents continued

Systems Biology — Cell-Free Protein Synthesis

EasyXpress — the fast and easy way to produce proteins



Typical yields from a single large-scale kit



Fast procedures — from gene to protein in a single day

Producing proteins used to be difficult and time-consuming. Not any more. QIAGEN's EasyXpress is the fast and easy way to produce proteins.

By using EasyXpress™ Kits you get:

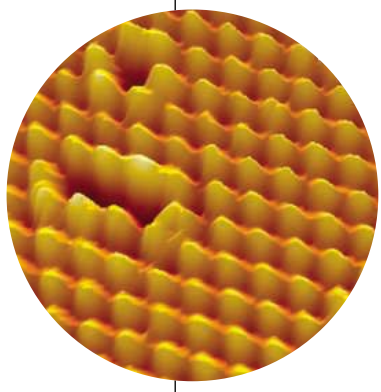
- **Efficient in vitro synthesis** — of native and posttranslationally modified eukaryotic proteins
- **Application-specific labeling** — for screening, protein interaction, and structural studies
- **Scalable, high yields** — a range of kits offering 3 µg to 5 mg of protein per reaction
- **Fast procedures and easy handling** — pure protein in hours, compared to weeks using conventional methods

To find out how fast and easy producing protein can be, visit www.qiagen.com/EasyXpress !

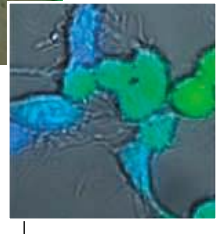
QIAGEN

Perspectives

Science Express www.sciencexpress.org



713 & 752



Brevia

Research Articles

741

Reports

Contents continued

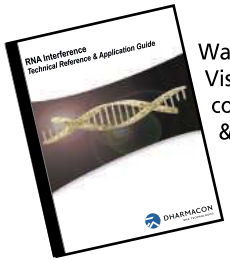
Advancing RNAi Technology.

Dharmacon...the world's most trusted siRNA resource

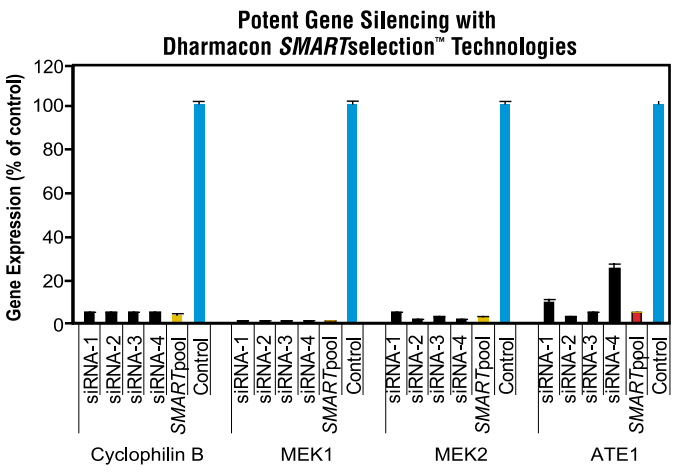
- The largest and most referenced siRNA supplier
- Target any unique human, mouse or rat gene
- Highest level of guaranteed silencing available
- Innovative technologies to enhance specificity
- Breakthrough siRNA transfection reagents
- Expert technical support

Leading RNAi researchers count on Dharmacon's state-of-the-art *SMART*selection™ and *SMART*pool® technologies for potent and specific gene silencing. Four individual siRNAs and a *SMART*pool siRNA reagent are available for over 66,000 unique human, mouse, and rat genes - each with the industry's best performance guarantee and backed by our expert technical support. Simply use our on-line si*GENOME*™ search tool to identify the siRNA reagents for your target gene.

No wonder Dharmacon is the most frequently referenced siRNA supplier in peer-reviewed journals!



Want to know more about RNAi? Visit our website to request your FREE copy of the RNAi Technical Reference & Application Guide today.



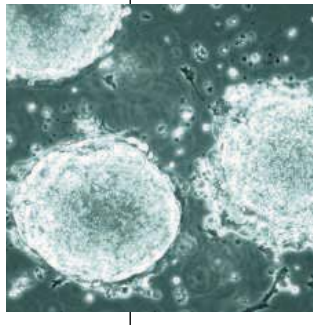
DHARMACON
RNA TECHNOLOGIES

www.dharmacon.com
Tel: 303-604-9499 or 800-235-9880 Fax: 303-604-9680

For European offices and distributors, please visit www.perbio.com
PERBIO

REPORTS CONTINUED

679 & 761



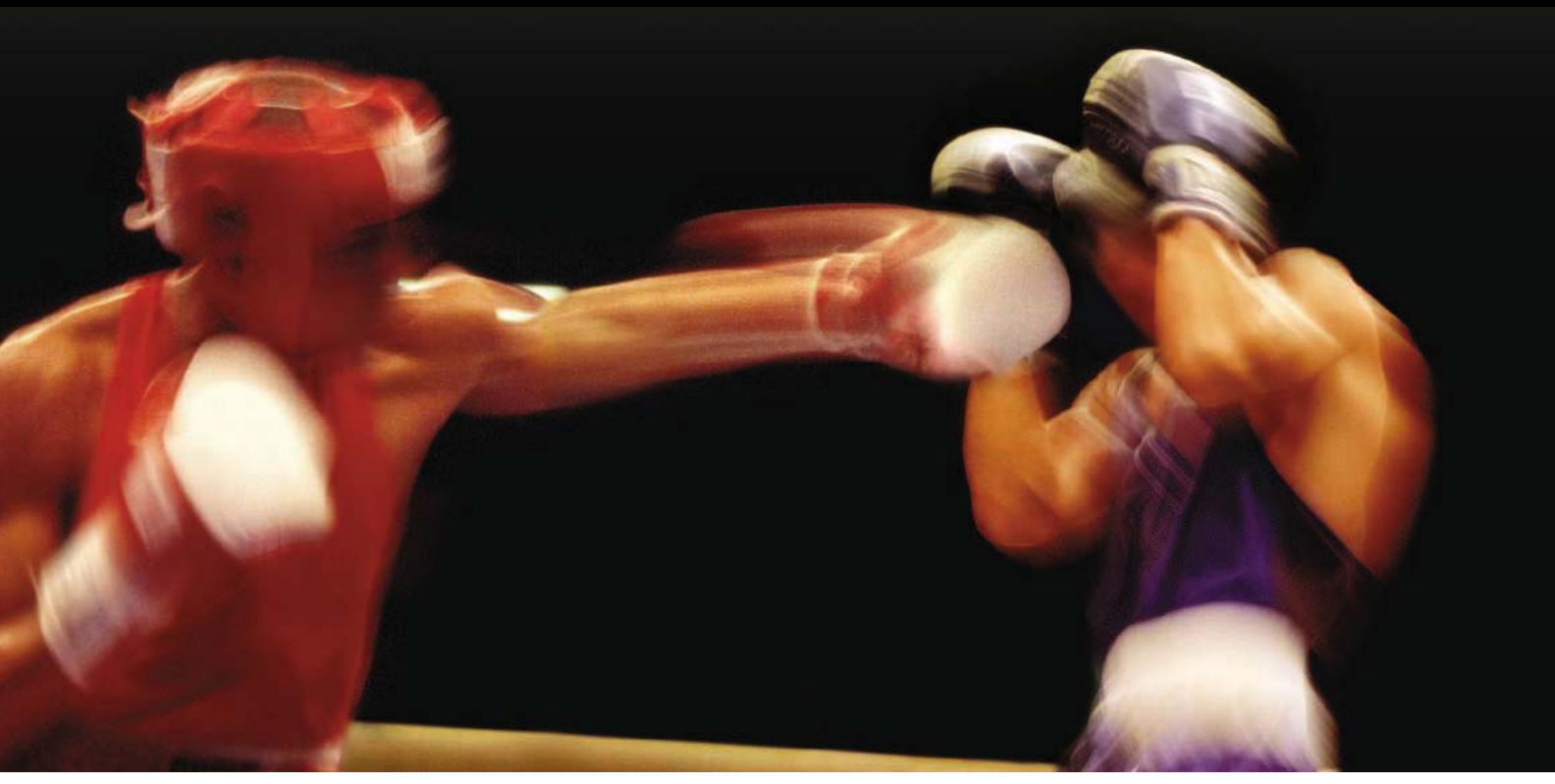
768

AAAS
ADVANCING SCIENCE, SERVING SOCIETY

SCIENCE (ISSN 0036-8075) is published weekly on Friday, except the last week in December, by the American Association for the Advancement of Science, 1200 New York Avenue, NW, Washington, DC 20005. Periodicals Mail postage (publication No. 484460) paid at Washington, DC, and additional mailing offices. Domestic individual membership and subscription (51 issues): $135 ($74 allocated to subscription). Domestic institutional subscription (51 issues): $550; Foreign postage extra: Mexico, Caribbean (surface mail) $55; other countries (air assist delivery) $85. First class, airmail, student, and emeritus rates on request. Canadian rates with GST available upon request, GST #1254 88122. Publications Mail Agreement Number 1069624. **Printed in the U.S.A.**

Change of address: allow 4 weeks, giving old and new addresses and 8-digit account number. **Postmaster:** Send change of address to *Science*, P.O. Box 1811, Danbury, CT 06813–1811. **Single copy sales:** $10.00 per issue prepaid includes surface postage; bulk rates on request. **Authorization to photocopy** material for internal or personal use under circumstances not falling within the fair use provisions of the Copyright Act is granted by AAAS to libraries and other users registered with the Copyright Clearance Center (CCC) Transactional Reporting Service, provided that $15.00 per article is paid directly to CCC, 222 Rosewood Drive, Danvers, MA 01923. The identification code for *Science* is 0036-8075/83 $15.00. *Science* is indexed in the *Reader's Guide to Periodical Literature* and in several specialized indexes.

Contents continued

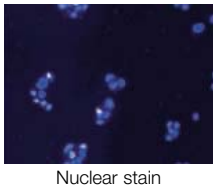


Knockout Performance

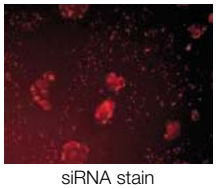
Powerful siRNA delivery starts with siLentFect™ lipid reagent for RNAi, the most efficient, flexible transfection reagent available.

- Low cytotoxicity reduces bias
- Excellent gene-specific knockdown obtained using as little as 5 nM siRNA
- Achieve >90% knockdown of both high- and low-abundance gene targets
- Simultaneously deliver siRNA and DNA for cotransfection applications

RNAi: A Bio-Rad pathway from delivery to detection. For more information, visit us on the Web at **www.bio-rad.com/ad/siLentFect/**



Nuclear stain



siRNA stain

Delivery of siRNA in MCF-7 cells. Cells were transfected with 10 nM siGLO siRNA using 0.5 µl siLentFect. After 24 hr, cells were imaged to show nuclear staining by Hoechst 33342 dye (top) or the location of fluorescent siRNA (bottom).

sciencenow www.sciencenow.org Daily News Coverage

When Good Clones Go Bad
Why have many of Dolly's successors been underachievers?

No Candy for Kitty
Cats don't seek out sugar because they can't taste it.

Atlantis Rises Again
A new sea-floor analysis reveals that a sunken landmass could have been the fabled island.

Industry as a research career choice.

science's next wave www.nextwave.org Career Resources for Young Scientists

Related Drug Discovery section page 721

▶ **Global/MiSciNet: Testing the Waters of Pharmaceutical Research** *R. Arnette*
Michael King is the associate director of clinical drug evaluation at Johnson & Johnson.

Global: Preparing for a Career in Industrial Research—Feature Index *R. Arnette*
Next Wave explores how to have a successful career in industrial research.

Global/US: Training and Transitions *D. Jensen*
The technical and interpersonal skills prized by industry are not often taught in traditional science-training programs.

Global/US: More Than Skin Deep *J. Kling*
A unique university-company partnership gives students a glimpse of life in the corporate fast lane.

Global/UK: A Step Inside Industry *A. Forde*
The UK's Industrial CASE Awards give doctoral candidates a taste of industry.

Global/EU: Keeping Both Academia and Industry on the Go *E. Pain*
An Italian scientist splits his time between a small company and a university.

science's sage ke www.sageke.org Science of Aging Knowledge Environment

News Focus: How Long Do I Have, Doc? *M. Leslie*
Protein foretells life span of genetically identical nematodes.

News Focus: Fat-Free Longevity *R. J. Davenport*
Mutation spurs fat accumulation and longevity through separate paths.

Heterogeneity in genetically identical individuals.

The quest for better medicines.

science's stke www.stke.org Signal Transduction Knowledge Environment

Related Drug Discovery section page 721

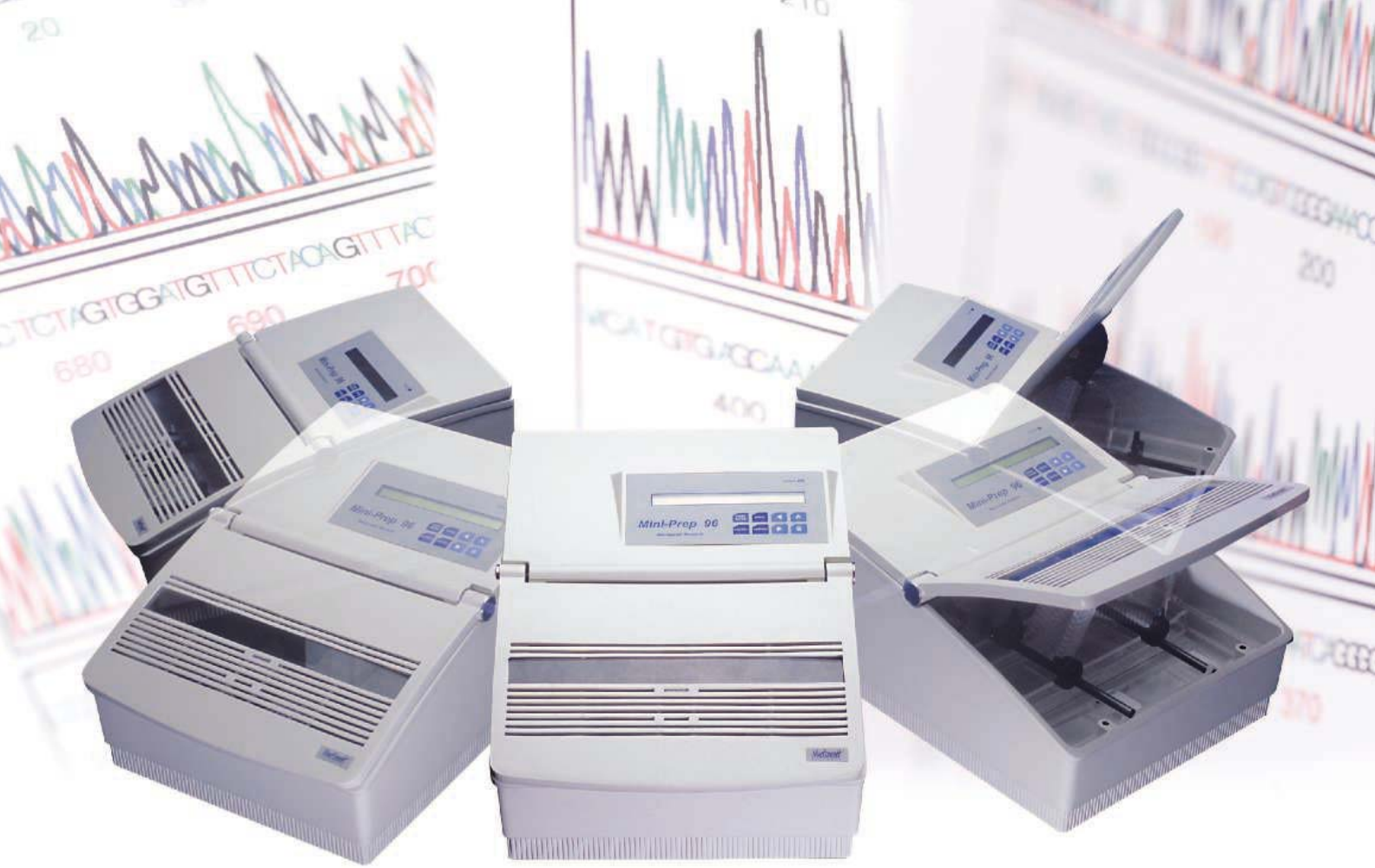
▶ **Editorial Guide: Focus Issue—Drug Discovery** *N. R. Gough*
Genetics, RNAi, and systems biology reveal new targets for therapeutic intervention.

▶ **Perspective: How Will RNAi Facilitate Drug Development?** *S. Bartz and A. L. Jackson*
RNAi may be used in multiple steps in drug target identification.

▶ **Perspective: Embracing Complexity, Inching Closer to Reality** *E. E. Schadt, A. Sachs, S. Friend*
Integrating high-throughput functional genomic and genotypic data with clinical trait data can elucidate signaling pathways associated with common human diseases.

▶ **Review: TRP Channels in Disease** *B. Nilius, T. Voets, J. Peters*
Understanding the genetics of disease may allow development of new therapeutic agents.

Separate individual or institutional subscriptions to these products may be required for full-text access.



Traditional Mini Preps are Over.

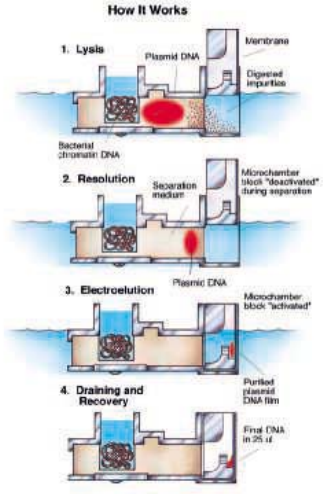
START ENTER

Start automating with the latest in plasmid DNA purification. The Mini Prep 96 can perform up to 96 preps in 1 hour of processing time. Up to 8 µg of plasmid DNA per lane at less than $1 a prep.

Start with easy operation. Disposable cassettes allow for direct loading of culture with no centrifugation.

Start the Mini Prep 96 with the push of a button. Remove high purity DNA and use in most microbiology protocols — including sequencing and cell transfection.

Start saving time and money with the Mini Prep 96.



Four Easy Steps to Plasmid DNA Purification

Stop Manual Mini Preps START ENTER Start the Mini Prep 96™

1-800-466-7949
www.macconnell.com

Mini-Preps at the Push of a Button.
6195 Cornerstone Court, San Diego, California 92121, Fax: 858-452-6753

MacConnell Research

Powerful Gamma Rays from X-ray Binaries

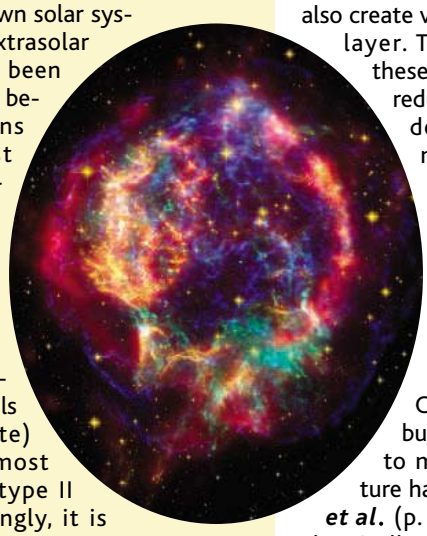
Active galactic nuclei (AGN), the very bright central regions of galaxies thought to be powered by matter falling into a black hole, are among the most energetic objects in the universe, and often exhibit jets of matter expanding at relativistic velocities. Although a million times less massive, x-ray binaries (a star orbiting a neutron star or black hole) can also show powerful outflows. These objects, called microquasars, appear to be smaller siblings of AGN. **Aharonian *et al.*** (p. 746, published online 7 July 2005; see the Perspective by **Cui**) report the detection of very high energy γ rays from an x-ray binary. Such γ emission is considered a key signature of jets in AGN. These results suggest a possible kinship between these two powerful classes of astrophysical objects.

Quantum Logic Spectrosopy

Precision spectroscopy of atoms usually involves laser-cooling, initial state preparation via optical pumping, and, after interrogation, internal-state detection of the atom. The atomic species generally used have been those that can be readily laser cooled, interrogated, and detected, but often at the expense of compromising the desirable spectroscopic property of narrow linewidth. **Schmidt *et al.*** (p. 749; see the Perspective by **Peik**) now show that these requirements can be fulfilled by using an auxiliary atomic species and quantum-logic techniques. This approach frees up the choice of the spectroscopy atom, including those whose spectroscopic transitions could serve, for example, as accurate atomic clocks.

Extrasolar Olivine

Meteorites and interplanetary dust particles (IDPs) can contain a few minerals and grains with isotopic compositions distinct from those found in our own solar system. Examples of extrasolar silicate grains have been few, however, in part because silicate grains are also the most common type in meteorites and IDPs. **Messenger *et al.*** (p. 737, published online 30 June 2005) have now identified such a grain composed of an aggregate of olivine crystals (an iron-rich silicate) from an IDP that most likely formed in a type II supernova. Surprisingly, it is still crystalline, which implies that this IDP spent only a few million years in the interstellar medium before our solar system formed.



Lining Up Vacancies

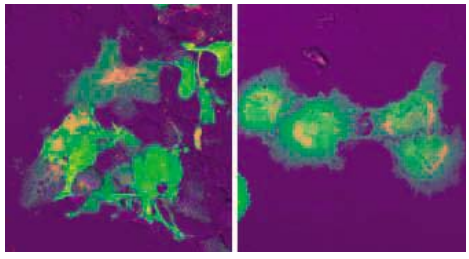
In a number of redox reactions catalyzed by noble metals at high temperatures, cerium oxide (CeO_2) is used as a support material because it can release and store oxygen. **Esch *et al.*** (p. 752; see the Perspective by **Campbell and Peden**) examined this process on the (111) surface of a CeO_2 crystal via high-resolution scanning tunneling microscopy and density functional calculations. When oxygen is released, the surface localizes the electrons through the reduction of Ce^{4+} to Ce^{3+}. The vacancies form lines of defects that expose the reduced Ce^{3+} ions, and these multiple defects also create vacancies in the subsurface layer. The initial formation of these structures demand more reducing equivalents than the desorption of a single O_2 molecule can provide, which may account for increased oxygen release when CeO_2 is doped with nonreducible Zr^{4+}.

Switching the Channel

One promising method for building nanoscale devices is to modify structures that nature has already produced. **Koçer *et al.*** (p. 755) prepared a photochemically gated valve by modifying the large conductance mechanosensitive channel protein, MscL, found in *Escherichia coli* cell membranes. The native protein functions as a pressure-relief valve and has a 3-nanometer pore. The authors modified a cysteine residue so that it undergoes charge separation upon ultraviolet irradiation. This charge-separated state permits ion flow through the otherwise hydrophobic channel, as evidenced in single molecule patch-clamp conduction studies.

The Taste of Things

Tastes can evoke emotional and behavioral responses and may be compared with memories of past encounters with the same food. **Sugita and Shiba** (p. 781) used transgenic expression of a transneuronal tracer to delineate the gustatory pathways within the brain of mice. The neuronal circuitries that process and integrate the information concerning the different taste qualities, such as bitter versus sweet, were segregated, which may provide the neuronal bases of taste discrimination, contrastive behavioral responses, and emotional states.



CREDITS (TOP TO BOTTOM): O. KRAUSE/NASA/JPL-CALTECH; SUGITA AND SHIBA

Designed for Robust Rice Production

Most agriculturally important traits, like grain number and plant height, are regulated by genes known as quantitative trait loci (QTLs) derived from natural allelic variations. Genetic crosses of existing rice lines allowed **Ashikari *et al.*** (p. 741, published online 23 June 2005) to identify several important QTLs involved in rice yield. One of these QTLs was identified as a candidate gene encoding a cytokinin oxidase. The locus was shown to encode a functional enzyme that degrades the hormone cytokinin. With less cytokinin degradation comes greater seed production, but also heavier panicles that are more susceptible to damage in the field. Combining the gene favoring greater grain production with a gene favoring shorter plants generated a significantly improved rice plant.

CONTINUED ON PAGE 667

Roche Applied Science

Only Two Steps To Success!

FuGENE 6 Transfection Reagent makes a big splash by:

> ***Works well, fast procedures, and doesn't kill cells***
>
> ***It's great for CHOs and cells are much healthier***
>
> ***Provides higher efficiency in High Five insect cells than the recommended transfection reagent***
>
> ***Cos-1 cells >80% transfection efficiency***
>
> **Actual Customer Comments**

- **Saving time**
  Transfect with FuGENE 6 Reagent in just two easy steps by eliminating media changes.
- **Getting results quicker**
  Avoid having to test different reagents when transfecting new cell types. FuGENE 6 is proven to transfect more than 700 cell types. Visit our transfection database at **www.roche-applied-science.com/fugene**.
- **Leading to results you can trust**
  Obtain more meaningful physiological results, because of the reagent's exceptionally low cytotoxicity.
- **Extending your resources**
  Increase productivity and lower costs by using less reagent per transfection.

Superior transient or stable transfection is closer and cheaper than you think. No wonder FuGENE 6 Reagent is cited in more than 3400 papers!

For more information, visit www.roche-applied-science.com/fugene

NEW! **X-tremeGENE siRNA Transfection Reagent**

For more information, visit www.roche-applied-science.com/geneknockdown or contact your local representative.

Roche Diagnostics

Roche Diagnostics GmbH
Roche Applied Science
68298 Mannheim
Germany

CONTINUED FROM 665

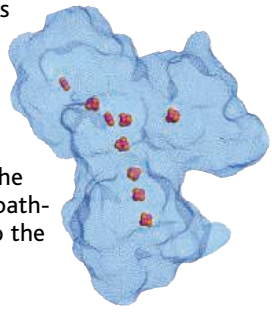
THIS WEEK IN Science

First Steps On All Fours

Fossil dinosaur eggs are fairly abundant, but finding embryos within them is rare. **Reisz *et al.*** (p. 761; see the news story by **Stokstad**) now have identified several embryos in eggs from South Africa dating to about 190 million years ago, much older than other dinosaur embryos. These embryos can be assigned to a common prosauropod thought to walk bipedally at times, but their forelimbs indicate that they hatched as quadrupeds. This difference raises the possibility that the later sauropods evolved by preservation of this early developmental state. The features of the hatchlings also suggest that they may have required parental care for some time.

Electron Transfer Structure Revealed

The last structural frontier in mitochondrial respiratory energetics is complex I. This membrane enzyme is the site where the high-energy electrons of reduced form of nicotinamide adenine dinucleotide (NADH) enter the series of mitochondrial complexes to drive adenosine triphosphate synthesis. The bacterial counterpart is a simpler grouping of 14 subunits, of which seven form the cytoplasmic domain where NADH is oxidized. **Hinchliffe and Sazanov** (p. 771) have dissociated and crystallized this seven-subunit assembly and determined the relative locations of the nine iron-sulfur clusters that provide an electron transfer pathway, 84 angstroms in length, from the NADH binding site to the proton-pumping domain.



CREDIT: HINCHLIFFE AND SAZANOV

Act On Your Senses

When a pathogen enters its host, it sets off an intruder alert system that ultimately mobilizes an immune attack force to deal with the offender. Is the host immune system perceived and responded to by the invader, just as a burglar might take evasive action upon hearing an alarm? **Wu *et al.*** (p. 774) find that *Pseudomonas aeruginosa*, a common bacterial pathogen of lung and intestine, does just that. By using a cell surface protein to bind the host cytokine, interferon-γ, the bacterium switches on at least two genes involved in the quorum-sensing system that governs growth and virulence within the host.

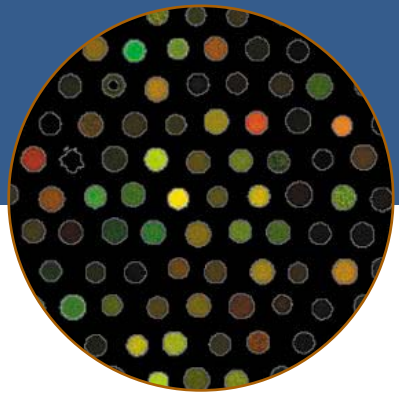
Squeezing Through the Pore

How proteins, which are composed of both hydrophobic and hydrophilic amino acid residues, are translocated across hydrophobic lipid bilayers has been the subject of intense scrutiny. The protective antigen component of anthrax toxin forms a homoheptameric pore in the target cell's endosomal membrane that creates a narrow passageway for the enzymatic components of the toxin to enter the cytosol. **Krantz *et al.*** (p. 777; see the Perspective by **von Heijne**) report that a set of seven closely apposed Phe residues in the aqueous lumen of the protective antigen pore is essential for its ability to translocate the other enzymatic subunits of anthrax toxin across the membrane. The "ϕ-clamp" appears to be the major conductance-blocking site for hydrophobic drugs and model cations and may serve a chaperone-like function in protein translocation.

Beyond Pavlov

It is relatively easy to transfer the physiological response to food (salivation) to a ringing bell when the stimuli are paired repeatedly. It also is possible to extinguish this association (or conditioning) if these stimuli are then presented in an unpaired fashion. Some associations appeared to be prepared or innate; a fearful response is more readily linked to seeing snakes rather than birds and is more difficult to extinguish. **Olsson *et al.*** (p. 785; see the Perspective by **Öhman**) now show that a conditioned fear response to faces from a social group different than one's own is more resistant to extinction than a similarly conditioned fear response to faces from one's own social group. This bias appears to be less in individuals with greater experience of the social out-group.

More Published



INSTRUMENTS

SOFTWARE

INFORMATICS

In a recent PubMed survey, more publications referenced GenePix® from Molecular Devices than any other slide-based microarray scanner platform. Why? Because researchers trust the results they get with GenePix scanners and Acuity® analysis software. When you trust the tools you use, you have the confidence to publish your results.

Obtaining reliable data requires high performance instruments and software that are easy to use and increase your productivity. With Molecular Devices' microarray scanning solutions, we give you that and more—so you can get more published.

Microarray Scanning

- GenePix Autoloader 4200AL: 36-slide capacity with automation
- GenePix Professional 4200A: four-laser flexibility and upgradeability
- GenePix 4000B: fastest two-laser scanner in existence
- GenePix Personal 4100A: affordable, quality two-laser scanning

Microarray Analysis Software

- GenePix Pro 6.0 microarray image analysis software
- Acuity 4.0 microarray informatics software

For more information, visit our web site or see us at Drug Discovery & Technology 2005, Booth #1113.

Molecular Devices

tel. 800-635-5577 | www.moleculardevices.com

Sending Pharma Better Signals

It's time to reassess what drives the discovery of new drugs. In its advertisements, one pharmaceutical company links innovation directly to its revenues: "Today's medicines finance tomorrow's miracles." If that formula really worked, we would have long since entered the golden age of therapeutics. After all, the pharmaceutical industry has been one of the most profitable businesses in America for years. Yet the number of new drugs emerging from most major pharmaceutical companies has been disappointing. What's wrong and how could things go better?

From one narrow perspective, nothing is wrong. These companies are investor-owned, publicly traded entities whose main responsibility is to provide shareholders with an optimal return on their investment. For most of the past 15 years, they have done a very good job at this, responding to signals sent from the marketplace. However, those signals often lead industry priorities in a direction that is lucrative but not well aligned with the health needs of the public. For example, the patent laws usually allow a company bringing a final product to market to keep all the marbles, often shutting out the upstream basic research on which those products are based. Those same laws also guarantee a brand-new patent to a manufacturer that makes a trivial change in an existing molecule, even if the "new" drug has the same clinical effect.

The U.S. Food and Drug Administration (FDA), for its part, sends forth only a weak signal. Approval is frequently granted if a new drug is merely better than a placebo at improving a surrogate measure in brief, modest-sized clinical trials. The agency rarely comments on the therapeutic importance of a new drug and never on its cost-effectiveness. Clinical trials comparing a new drug with existing treatments are typically required only when placebo controls are ethically unacceptable. Other agencies disdain funding such studies or lack the resources to do so. Large payors inside and outside the government hardly ever mount the comparative trials whose results could be so valuable to them. Physicians also bear responsibility for these degraded marketplace signals by relying too heavily on promotional information and company-sponsored education to drive prescribing decisions. Direct-to-consumer advertising now enlists patients as well in this triumph of marketing over science.

The ultimate market signal—dollars—comes from the country's health care payors. With the notable exception of the U.S. Department of Veterans Affairs and a few large health maintenance organizations, most payors in both the public and private sectors willingly, if complainingly, pay for whatever doctors prescribe and companies charge, however unremarkable a drug's therapeutic value or cost-effectiveness. This particular signal is likely to become even more problematic in January 2006, when Medicare begins paying for outpatient drugs, because the new benefit prohibits the government from considering these issues.

How can we change these noise-laden signals into a message that could foster more useful pharmaceutical innovation? We can start by using patent laws to increase rewards for the basic science that undergirds so much of what the industry does. Those laws could also take a more conservative view of whether a company's one-atom changes or isomerization of an existing molecule warrant monopoly protection. The FDA could require more useful and demanding pre-marketing studies and ask its advisory committees to comment on whether a newly approved drug is an important therapeutic contribution or an unremarkable addition to an already-full class. Prescribing physicians could focus more on actual clinical trial data and refuse to help sell a drug just because it has a zippy marketing campaign. And patients could learn that advertisements are not the best measure of a medicine's therapeutic value. Payors inside and outside government could make purchasing decisions based solely on critical reviews of the clinical and economic evidence.

Marketplace solutions are by no means a panacea. They will never be adequate to foster the development of drugs for which the market is too poor or too small to generate a profit. But for the major common diseases of the developed world, these changes could help reform and rescue an industry trapped by its own clever marketing successes. Major change will have to come from inside the large pharmaceutical manufacturers as well. Presenting them with more intelligent incentives would help move them along the right path. Those companies are adept at responding to signals; we need to send them the right ones.

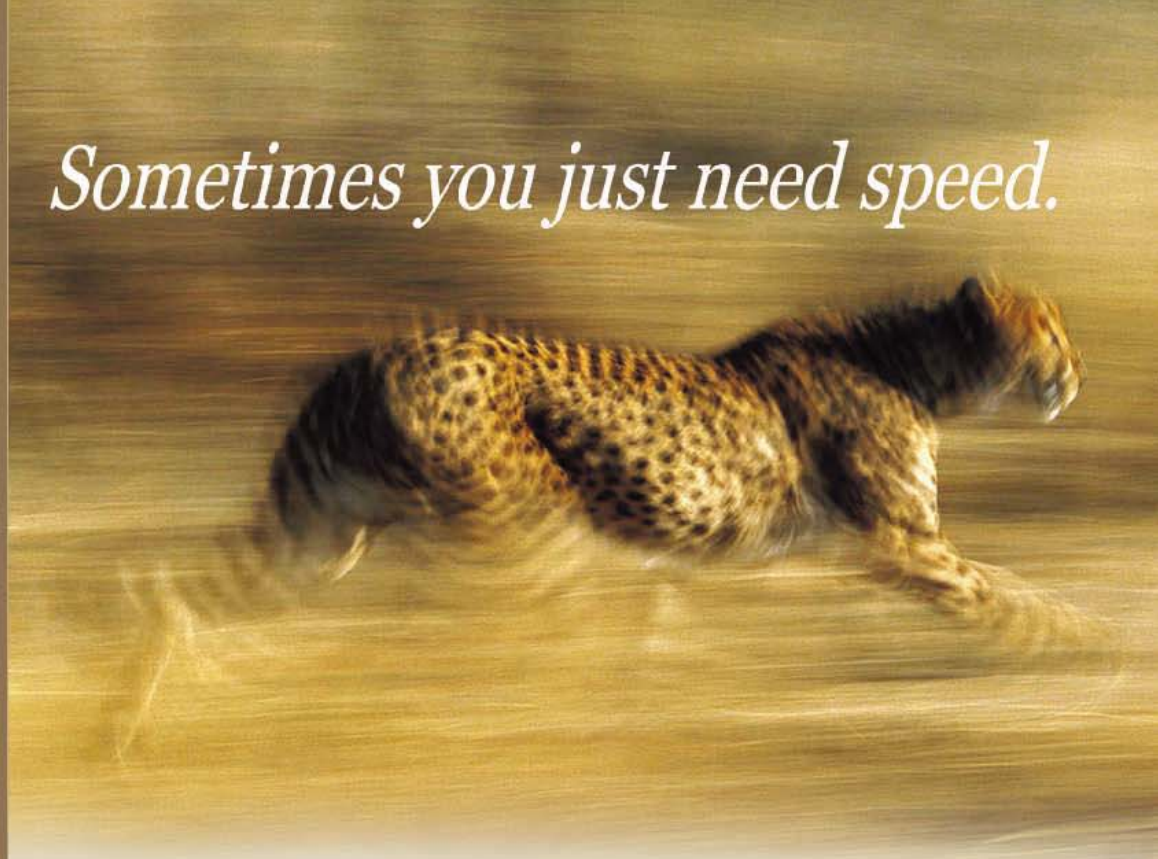
Jerry Avorn

Jerry Avorn is a professor of medicine at Harvard Medical School and chief of the Division of Pharmacoepidemiology and Pharmacoeconomics at the Brigham and Women's Hospital.

10.1126/science.1117448

SameDay Oligos

Sometimes you just need speed.



SameDay Oligo Specifications:

- **Order via the Web by 1:00 P.M. CST today**
- **Receive the oligos next business morning via priority shipping!!**
- **25-nmole scale**
- **2-OD guarantee (sufficient for > 250 PCR reactions)**
- **15-45 bases**
- **Deprotected & desalted**
- **Unmodified**
- **Available within the U.S. and Canada**

Innovation & Precision in Nucleic Acid Synthesis

1710 Commercial Park
Coralville, IA 52241 USA
800.328.2661 fax: 319.626.8444
www.idtdna.com

IDT
INTEGRATED DNA TECHNOLOGIES, INC.

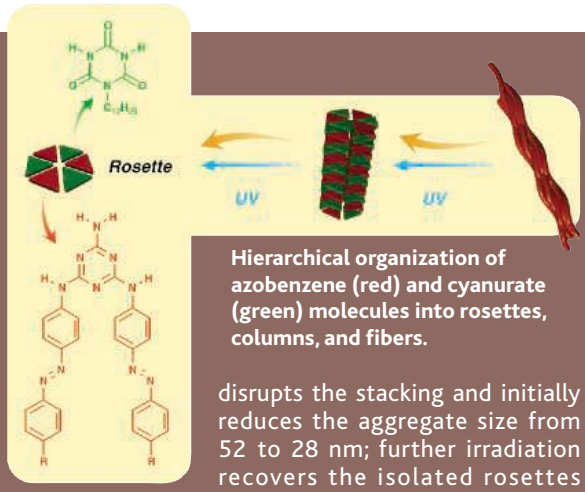
EDITORS' CHOICE

edited by Gilbert Chin

CHEMISTRY

Lightly Switched Gel

The formation of supramolecular assemblies can be controlled through light-induced structural movements, such as cis-trans isomerization, that alter the interactions between weakly bonding molecules. Yagai *et al.* have characterized disc-shaped hydrogen-bonded hexamers (rosettes) formed from two molecules: one a melamine bearing two long side chains containing azo groups and the other a much smaller cyanurate. In cyclohexane solution, the rosettes formed from the *trans*-azobenzene isomer can stack through aromatic interactions and bunch into columns that eventually intertwine and gel. Irradiation of the gel with ultraviolet light disrupts the stacking and initially reduces the aggregate size from 52 to 28 nm; further irradiation recovers the isolated rosettes (8-nm aggregates). The dissociation is reversible, and exposure to visible light and subsequent storage in the dark yields the gel with total conversion of the cis isomers back to *trans*-azobenzenes. — PDS



Hierarchical organization of azobenzene (red) and cyanurate (green) molecules into rosettes, columns, and fibers.

J. Am. Chem. Soc. 10.1021/ja052645a (2005).

PHYSICS

Cold Atom Coupling

The ability to control the interaction strength between atoms within strongly interacting Fermi gases by sweeping a magnetic field across a Feshbach resonance provides a powerful experimental system in which to study many-body physics. One example is the crossover from a Bose-Einstein condensate (BEC) regime, in which the atoms are strongly coupled into pairs, to the weak-coupling regime that mimics Bardeen-Cooper-Schrieffer (BCS) coupling of electrons in superconducting metals. Although behavior on either side of the resonance is fairly well understood, of immediate interest is to find out what happens in the BEC-BCS crossover regime. However, determining the relative contributions of atom pairing mechanisms is an experimental and theoretical challenge.

Partridge *et al.* use a molecular spectroscopy technique to probe how the atoms pair up near the resonance. A laser is used to dress pairs of atoms and project them onto a known molecular energy level. Locking the excitation rate onto the molecular level allows them to make a precise measurement of the contribution of each pairing mechanism. The technique should prove useful for closer studies of the many-body physics involved in these cold atom systems. — ISO

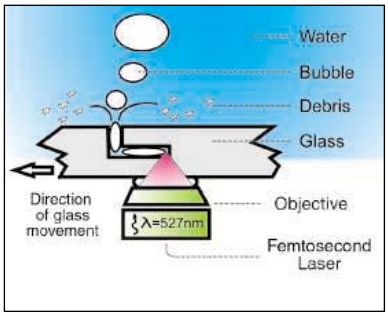
Phys. Rev. Lett. **95**, 020404 (2005).

CHEMISTRY

A Bit of Bubbly

The popularity of the rapidly advancing field of microfluidics is due in part to the simplicity of making parts from polymers through etching or patterning methods. Some of the limitations of the commonly used polydimethylsiloxane are solvent swelling, protein adsorption, leaching, and the inability to contain high pressures. Silica glass is often the best material for vessels for analytical and synthetic chemistry, but patterning glass at submicrometer dimensions is a challenge.

Ke *et al.* show that by using low-energy laser pulses, and by immersing the glass in a liquid, they can fabricate small channels in three dimensions. The laser is focused to a spot at the liquid/glass interface, so that a pulse both forms a hole in the glass and causes the liquid to expand as a bubble that pushes away the debris. Because the pulses are of low energy, the bubbles expand slowly and persist for much longer times than those associated with supersonic bubble collapse. The authors fabricated a number of architectures and channel designs, including a crisscross design that enhances the mixing of the fluids. — MSL



Schematic of the fabrication setup.

Anal. Chem. 10.1021/ac0505167 (2005).

BIOCHEMISTRY

An On-Off Cycle

The mechanisms by which the activities of regulatory enzymes are themselves regulated range from tight-binding inhibitors to covalent modification. Sivaramakrishnan *et al.* have used a small molecule model in order to explore the chemical feasibility of regulating protein tyrosine phosphatase 1B (PTP1B) by reversible oxidation of its catalytic sulfhydryl. Structural analysis of inhibited PTP1B revealed the presence of a 3-isothiazolidinone adduct, in which the side chain of the active site cysteine had become covalently linked to the amide nitrogen of the next residue. Using a benzene scaffold to juxtapose a β-sulfinyl propionic acid ester and a monosubstituted amide nitrogen, they find that the in situ–generated sulfenic acid (RS-OH) is sufficiently reactive for the heterocycle to form under mild conditions (pH 7.5 and 37°C). In terms of how the corresponding biochemistry occurs, hydrogen peroxide oxidizes the sulfhydryl to the sulfenic acid, and glutathione opens the ring, forming a mixed disulfide that regenerates the free sulfhydryl. These reactions together would then serve as a redox cycle, switching phosphatase activity on and off. — GJC

J. Am. Chem. Soc. 10.1021/ja052599e (2005).

IMMUNOLOGY

Sweet Relations

Although bacteria are often thought of as harmful, it is now recognized that the many bacteria species harbored by our intestines are essential for our well-being. Aside from their roles in eliminating toxins and extracting nutrients, there is much interest in understanding

CONTINUED ON PAGE 673

Genetic Engineering that is Right on Target!

The TargeTron™ Gene Knockout System is a revolutionary method for rapid and specific disruption of genes in prokaryotic organisms. Utility of the technology has been demonstrated for prokaryotic genetic engineering, systems biology and functional genomics approaches.

The method exploits the retrohoming ability of group II introns and utilizes a simple PCR step to "re-target" the TargeTron group II intron for specific insertion into the host genome. Gene knockout using the TargeTron system has been validated in a broad range of bacterial strains such as *Escherichia coli, Staphylococcus aureus, Lactococcus lactis, Clostridium perfringens, Shigella flexneri* and *Salmonella typhimurium.*

- Targeted and permanent gene disruption
- Simple, streamlined protocol; Knockouts in 3 days or less
- Minimal screening to isolate mutants.
- No cell conjugation or specific host factor requirements
- >90% successful targeted insertion

Product	Description	Unit
TA0100	TargeTron™ Gene Knockout System	3EA 10EA

For additional details and product availability, please visit www.sigma-aldrich.com/s1target

how the gut microflora might influence the development and function of our immune systems.

Building on previous work in which bacterial zwitterionic polysaccharides were shown to be presented as antigens in the activation of T cells, Mazmanian *et al.* observe that at least one such sugar—polysaccharide A (PSA)—can direct normal immune system development in the mouse. Reconstitution of germ-free mice with the bacterial commensal *Bacteroides fragilis* expanded T cell numbers and restored lymphoid structures that would otherwise have developed abnormally. Expression of PSA was sufficient and necessary for this activity and also reestablished balance in T helper 1 (T_H1) and T_H2 cell cytokine responses, through presentation of PSA by dendritic cells. The finding that a bacterial product can implement such direct governance over the mammalian immune system may explain how our microflora help maintain pathogen immunity while preventing unwanted inflammation and allergy. — SJS

Cell **122**, 107 (2005).

PSYCHOLOGY

On Being a Team Player

Participating in team sports, such as baseball, can bring into play an individual's competitive tendencies (vying for a starting position) even though cooperation, as in the execution of fundamental skills such as hitting behind the runner, may be needed for success at the highest level. Historically, statistical assessment has contrasted the relative achievements of players, particularly during contract negotiations, but recent analyses have used sophisticated approaches to quantify less tangible player contributions to team success, such as moving a runner into scoring position.

Stapel and Koomen have examined the influence of personal orientation (toward cooperation or competition) on an individual's evalution of self in relation to a target. They find that a cooperative mindset yielded an enhancement of one's self-evaluation relative to a high-achieving target—referred to as assimilation—whereas the same target attributes pushed downward the self-ratings of competitive subjects. Framing the target within a cooperative or competitive context either by manipulating the scenario explicitly or by activating goals implicitly were equally effective in influencing how subjects adjusted their self-appraisals upward or downward. Finally, these positive/negative shifts also applied to comparisons in which the same pair of photographs was labeled as more or less similar depending on whether the situation was deemed to be cooperative or competitive. — GJC

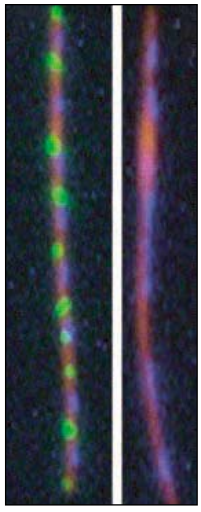
J. Pers. Soc. Psychol. **88**, 1029 (2005).

CELL BIOLOGY

Pole to Pole

Bacillus subtilis is a rod-shaped bacterium that is competent to bind, internalize, and eventually incorporate DNA in a process known as transformation. Hahn *et al.* describe the localization of three competence-mediating proteins and find that they are preferentially associated with the poles of the cells in a dynamic fashion. Using laser tweezers to manipulate single fluorescent DNA molecules, they observed that DNA binding and uptake occurs preferentially at the poles, too.

Kidane and Graumann also examine protein and DNA dynamics in *B. subtilis*. They find that the DNA recombination enzyme RecA colocalizes at the cell poles with one of the competence proteins, and during DNA uptake formed into threads. In comparison, another DNA recombination protein, RecN, was observed to oscillate from pole to pole on the scale of minutes; however, when DNA was added, RecN arrested at the same end where competence proteins were located, due to direct interaction with incoming DNA. The dynamic assembly and disassembly of the competence machinery are likely to govern exactly how transformable particular bacteria may be at a given time. — SMH



Filaments of *B. subtilis* (red) expressing (left) or not expressing (right) competence proteins (green) localized between nucleoids (blue).

Cell **122**, 59; 73 (2005).

CREDITS: HAHN *ET AL.*, *CELL* 122, 59 (2005)

Turn to page 793

www.sciencemag.org

Science

1200 New York Avenue, NW
Washington, DC 20005
Editorial: 202-326-6550, FAX 202-289-7562
News: 202-326-6500, FAX 202-371-9227

Bateman House, 82-88 Hills Road
Cambridge, UK CB2 1LQ
+44 (0) 1223 326500, FAX +44 (0) 1223 326501

SUBSCRIPTION SERVICES For change of address, missing issues, new orders and renewals, and payment questions: 800-731-4939 or 202-326-6417, FAX 202-842-1065. Mailing addresses: AAAS, P.O. Box 1811, Danbury, CT 06813 or AAAS Member Services, 1200 New York Avenue, NW, Washington, DC 20005

INSTITUTIONAL SITE LICENCES please call 202-326-6755 for any questions or information

REPRINTS Ordering/Billing/Status 800-635-7171; Corrections 202-326-6501

PERMISSIONS 202-326-7074, FAX 202-682-0816

MEMBER BENEFITS Bookstore: AAAS/BarnesandNoble.com bookstore www.aaas.org/bn; Car purchase discount: Subaru VIP Program 202-326-6417; Credit Card: MBNA 800-847-7378; Car Rentals: Hertz 800-654-2200 CDP#343457, Dollar 800-800-4000 #AA1115; AAAS Travels: Betchart Expeditions 800-252-4910; Life Insurance: Seabury & Smith 800-424-9883; Other Benefits: AAAS Member Services 202-326-6417 or www.aaasmember.org.

science_editors@aaas.org (for general editorial queries)
science_letters@aaas.org (for queries about letters)
science_reviews@aaas.org (for returning manuscript reviews)
science_bookrevs@aaas.org (for book review queries)

Published by the American Association for the Advancement of Science (AAAS), *Science* serves its readers as a forum for the presentation and discussion of important issues related to the advancement of science, including the presentation of minority or conflicting points of view, rather than by publishing only material on which a consensus has been reached. Accordingly, all articles published in *Science*—including editorials, news and comment, and book reviews—are signed and reflect the individual views of the authors and not official points of view adopted by the AAAS or the institutions with which the authors are affiliated.

AAAS was founded in 1848 and incorporated in 1874. Its mission is to advance science and innovation throughout the world for the benefit of all people. The goals of the association are to: foster communication among scientists, engineers and the public; enhance international cooperation in science and its applications; promote the responsible conduct and use of science and technology; foster education in science and technology for everyone; enhance the science and technology workforce and infrastructure; increase public understanding and appreciation of science and technology; and strengthen support for the science and technology enterprise.

INFORMATION FOR CONTRIBUTORS

See pages 135 and 136 of the 7 January 2005 issue or access www.sciencemag.org/feature/contribinfo/home.shtml

EDITOR-IN-CHIEF **Donald Kennedy**
EXECUTIVE EDITOR **Monica M. Bradford**
DEPUTY EDITORS **R. Brooks Hanson, Katrina L. Kelner**
NEWS EDITOR **Colin Norman**

EDITORIAL SUPERVISORY SENIOR EDITORS Barbara Jasny, Phillip D. Szuromi; **SENIOR EDITORS** Gilbert J. Chin, Lisa D. Chong, Pamela J. Hines, Paula A. Kiberstis (Boston), Beverly A. Purnell, L. Bryan Ray, Guy Riddihough (Manila), H. Jesse Smith, Valda Vinson, David Voss; **ASSOCIATE EDITORS** Marc S. Lavine, Jake S. Yeston; **ONLINE EDITOR** Stewart Wills; **CONTRIBUTING EDITOR** Ivan Amato; **ASSOCIATE ONLINE EDITOR** Tara S. Marathe; **BOOK REVIEW EDITOR** Sherman J. Suter; **ASSOCIATE LETTERS EDITOR** Etta Kavanagh; **INFORMATION SPECIALIST** Janet Kegg; **EDITORIAL MANAGER** Cara Tate; **SENIOR COPY EDITORS** Jeffrey E. Cook, Harry Jach, Barbara P. Ordway; **COPY EDITORS** Cynthia Howe, Alexis Wynne Mogul, Sabrah M. n'haRaven, Jennifer Sills, Trista Wagoner; **EDITORIAL COORDINATORS** Carolyn Kyle, Beverly Shields; **PUBLICATION ASSISTANTS** Chris Filiatreau, Joi S. Granger, Jeffrey Hearn, Lisa Johnson, Scott Miller, Jerry Richardson, Brian White, Anita Wynn; **EDITORIAL ASSISTANTS** Ramatoulaye Diop, E. Annie Hall, Patricia M. Moore, Brendan Nardozzi, Michael Rodewald; **EXECUTIVE ASSISTANT** Sylvia S. Kihara; **ADMINISTRATIVE SUPPORT** Patricia F. Fisher

NEWS SENIOR CORRESPONDENT Jean Marx; **DEPUTY NEWS EDITORS** Robert Coontz, Jeffrey Mervis, Leslie Roberts, John Travis; **CONTRIBUTING EDITORS** Elizabeth Culotta, Polly Shulman; **NEWS WRITERS** Yudhijit Bhattacharjee, Jennifer Couzin, David Grimm, Constance Holden, Jocelyn Kaiser, Richard A. Kerr, Eli Kintisch, Andrew Lawler (New England), Greg Miller, Elizabeth Pennisi, Charles Seife, Robert F. Service (Pacific NW), Erik Stokstad; Carolyn Gramling, Genevra Ornelas, Cathy Tran (interns); **CONTRIBUTING CORRESPONDENTS** Marcia Barinaga (Berkeley, CA), Barry A. Cipra, Adrian Cho, Jon Cohen (San Diego, CA), Daniel Ferber, Ann Gibbons, Robert Irion, Mitch Leslie (NetWatch), Charles C. Mann, Evelyn Strauss, Gary Taubes, Ingrid Wickelgren; **COPY EDITORS** Linda B. Felaco, Rachel Curran, Sean Richardson; **ADMINISTRATIVE SUPPORT** Scherraine Mack, Fannie Groom **BUREAUS:** Berkeley, CA: 510-652-0302, FAX 510-652-1867, New England: 207-549-7755, San Diego, CA: 760-942-3252, FAX 760-942-4979, Pacific Northwest: 503-963-1940

PRODUCTION DIRECTOR James Landry; **SENIOR MANAGER** Wendy K. Shank; **ASSISTANT MANAGER** Rebecca Doshi; **SENIOR SPECIALISTS** Vicki J. Jorgensen, Jessica K. Moshell; **SPECIALISTS** Jay R. Covert, Stacey Ferebee; **PREFLIGHT DIRECTOR** David M. Tompkins; **MANAGER** Marcus Spiegler; **SPECIALIST** Jessie Mudjitaba;

ART DIRECTOR Joshua Moglia; **ASSOCIATE ART DIRECTOR** Kelly Buckheit; **ILLUSTRATOR** Katharine Sutliff; **SENIOR ART ASSOCIATES** Holly Bishop, Laura Creveling, Preston Huey, Julie White; **ASSOCIATE** Nayomi Kevitiyagala; **PHOTO RESEARCHER** Leslie Blizard

***SCIENCE* INTERNATIONAL**

EUROPE (science@science-int.co.uk) **EDITORIAL: INTERNATIONAL MANAGING EDITOR** Andrew M. Sugden; **SENIOR EDITOR/PERSPECTIVES** Julia Fahrenkamp-Uppenbrink; **SENIOR EDITORS** Caroline Ash (Geneva: +41 (0) 222 346 3106), Stella M. Hurtley, Ian S. Osborne, Peter Stern; **ASSOCIATE EDITOR** Stephen J. Simpson; **EDITORIAL SUPPORT** Emma Westgate; Deborah Dennison **ADMINISTRATIVE SUPPORT** Janet Clements, Phil Marlow, Jill White; **NEWS: INTERNATIONAL NEWS EDITOR** Eliot Marshall **DEPUTY NEWS EDITOR** Daniel Clery; **CORRESPONDENT** Gretchen Vogel (Berlin: +49 (0) 30 2809 3902, FAX +49 (0) 30 2809 8365); **CONTRIBUTING CORRESPONDENTS** Michael Balter (Paris), Martin Enserink (Amsterdam and Paris); **INTERN** Mason Inman

ASIA Japan Office: Asca Corporation, Eiko Ishioka, Fusako Tamura, 1-8-13, Hirano-cho, Chuo-ku, Osaka-shi, Osaka, 541-0046 Japan; +81 (0) 6 6202 6272, FAX +81 (0) 6 6202 6271; asca@os.gulf.or.jp **JAPAN NEWS BUREAU:** Dennis Normile (contributing correspondent, +81 (0) 3 3391 0630, FAX 81 (0) 3 5936 3531; dnormile@gol.com); **CHINA REPRESENTATIVE** Hao Xin, + 86 (0) 10 6307 4439 or 6307 3676, FAX +86 (0) 10 6307 4358; haoxin@earthlink.net; **SOUTH ASIA** Pallava Bagla (contributing correspondent +91 (0) 11 2271 2896; pbagla@vsnl.com); **CENTRAL ASIA** Richard Stone (+7 3272 6413 35, rstone@aaas.org)

EXECUTIVE PUBLISHER **Alan I. Leshner**
PUBLISHER **Beth Rosner**

FULFILLMENT & MEMBERSHIP SERVICES (membership@aaas.org) **DIRECTOR** Marlene Zendell; **MANAGER** Waylon Butler; **SYSTEMS SPECIALIST** Andrew Vargo **SENIOR SPECIALIST** Pat Butler; **SPECIALISTS** Laurie Baker, Tamara Alfson, Karena Smith

BUSINESS OPERATIONS AND ADMINISTRATION DIRECTOR Deborah Rivera-Wienhold; **BUSINESS MANAGER** Randy Yi; **SENIOR BUSINESS ANALYST** Lisa Donovan; **BUSINESS ANALYST** Jessica Tierney; **FINANCIAL ANALYST** Michael LoBue, Farida Yeasmin; **RIGHTS AND PERMISSIONS: ADMINISTRATOR** Emilie David; **ASSOCIATE** Elizabeth Sandler; **MARKETING: DIRECTOR** John Meyers; **MEMBERSHIP MARKETING MANAGER** Darryl Walter; **MARKETING ASSOCIATE** Julianne Wielga; **RECRUITMENT MARKETING MANAGER** Allison Pritchard; **ASSOCIATES** Mary Ellen Crowley, Amanda Donathen, Catherine Featherston; **DIRECTOR OF INTERNATIONAL MARKETING AND RECRUITMENT ADVERTISING** Deborah Harris; **INTERNATIONAL MARKETING MANAGER** Wendy Sturley; **MARKETING/MEMBER SERVICES EXECUTIVE:** Linda Rusk; **JAPAN SALES AND MARKETING MANAGER** Jason Hannaford; **SITE LICENSE SALES: DIRECTOR** Tom Ryan; **SALES AND CUSTOMER SERVICE** Mehan Dossani, Catherine Holland, Adam Banner, Yaniv Snir; **ELECTRONIC MEDIA: INTERNET PRODUCTION MANAGER** Lizabeth Harman; **ASSISTANT PRODUCTION MANAGER** Wendy Stengel; **SENIOR PRODUCTION ASSOCIATES** Sheila Mackall, Amanda K. Skelton, Lisa Stanford; **PRODUCTION ASSOCIATE** Nichele Johnston; **LEAD APPLICATIONS DEVELOPER** Carl Saffell

PRODUCT ADVERTISING (science_advertising@aaas.org); **MIDWEST** Rick Bongiovanni: 330-405-7080, FAX 330-405-7081 • **WEST COAST/W. CANADA** B. Neil Boylan (Associate Director): 650-964-2266, FAX 650-964-2267 • **EAST COAST/E. CANADA** Christopher Breslin: 443-512-0330, FAX 443-512-0331 • **UK/SCANDINAVIA/FRANCE/ITALY/BELGIUM/NETHERLANDS** Andrew Davies (Associate Director): +44 (0)1782 750111, FAX +44 (0) 1782 751999 • **GERMANY/SWITZERLAND/AUSTRIA** Tracey Peers (Associate Director): +44 (0) 1782 752530, FAX +44 (0) 1782 752531 **JAPAN** Mashy Yoshikawa: +81 (0) 33235 5961, FAX +81 (0) 33235 5852 **ISRAEL** Jessica Nachlas +9723 5449123 • **TRAFFIC MANAGER** Carol Maddox; **SALES COORDINATOR** Deiandra Simms

CLASSIFIED ADVERTISING (advertise@sciencecareers.org); **U.S.: SALES DIRECTOR** Gabrielle Boguslawski: 718-491-1607, FAX 202-289-6742; **INTERNET SALES MANAGER** Beth Dwyer: 202-326-6534; **INSIDE SALES MANAGER** Daryl Anderson: 202-326-6543; **WEST COAST/MIDWEST** Kristine von Zedlitz: 415-956-2531; **EAST COAST** Jill Downing: 631-580-2445; **LINE AD SALES** Emnet Tesfaye: 202-326-6740; **SENIOR SALES COORDINATOR** Erika Bryant; **SALES COORDINATORS** Rohan Edmonson, Christopher Normile, Joyce Scott, Shirley Young; **INTERNATIONAL: SALES MANAGER** Tracy Holmes: +44 (0) 1223 326525, FAX +44 (0) 1223 326532; **SALES** Christina Harrison, Suitlana Barnes; **SALES ASSISTANT** Helen Moroney; **JAPAN:** Jason Hannaford: +81 (0) 52 789 1860, FAX +81 (0) 52 789 1861; **PRODUCTION: MANAGER** Jennifer Rankin; **ASSISTANT MANAGER** Deborah Tompkins; **ASSOCIATE** Amy Hardcastle; **SENIOR TRAFFICKING ASSOCIATE** Christine Hall; **SENIOR PUBLICATIONS ASSISTANT** Robert Buck; **PUBLICATIONS ASSISTANT** Natasha Pinol

AAAS BOARD OF DIRECTORS RETIRING PRESIDENT, CHAIR Shirley Ann Jackson; **PRESIDENT** Gilbert S. Omenn; **PRESIDENT-ELECT** John P. Holdren; **TREASURER** David E. Shaw; **CHIEF EXECUTIVE OFFICER** Alan I. Leshner; **BOARD** Rosina M. Bierbaum; John E. Burris; John E. Dowling; Lynn W. Enquist; Susan M. Fitzpatrick; Richard A. Meserve; Norine E. Noonan; Peter J. Stang; Kathryn D. Sullivan

AAAS
ADVANCING SCIENCE, SERVING SOCIETY

Patience?

Patients can't afford it.

And, if you're like our scientists, you want to help them now. The good news is, you can. By partnering with Perlegen, you can initiate whole genome association studies that analyze millions of unique SNPs in hundreds of cases and controls.

If you have access to DNA samples for a well-characterized phenotype, we can help you impact patient care now. Our scientists will collaborate with you to perform, analyze, and publish whole genome association studies in a matter of months, not years.

Patients are waiting.

To learn more, contact:
Partnerships21@perlegen.com
www.perlegen.com

Targeting today's drugs.
Discovering tomorrow's.™

PERLEGEN SCIENCES®

Roche Applied Science

Genome Sequencer 20 System

First to the Finish

Revolutionize your research with the newest innovation in rapid whole genome sequencing and assembly — the new **Genome Sequencer 20** from Roche Applied Science. Perform genomic analyses not currently possible due to the cost and time constraints of current methods.

- **Generate data fast**
  Sequence at least 20 million bases in one 4.5 hour run.
- **Spend less per base**
  Reduce cost per raw base with rapid, efficient amplification and sequencing through picoliter format with massive parallelization.
- **Simplify sample preparation**
  Eliminate cloning and colony picking with single-tube library preparation per genome.
- **Expand your versatility**
  Perform a wide variety of projects with *de novo* assembly and mapping software.

Be first to the finish! Explore the power of the newest revolution in sequencing today — visit ***www.roche-applied-science.com/sis/sequencing/genome/*** or contact your local sales representative.

454 LIFE SCIENCES

Roche Diagnostics

Roche Diagnostics GmbH
Roche Applied Science
68298 Mannheim
Germany

NetWatch

edited by Mitch Leslie

FUN

Star Trekking

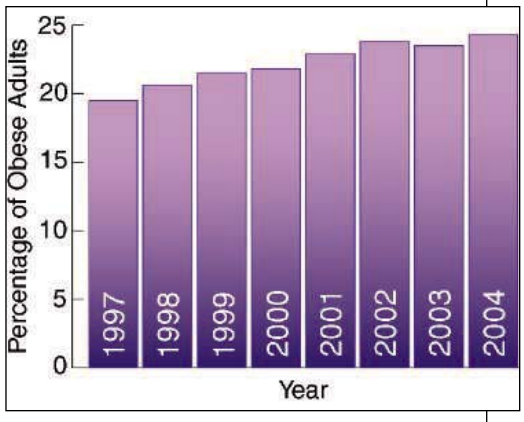
Tired of the unchanging view from your office window? Feeling trapped in the lab? Perhaps you need a quick excursion to Mars, where you can sidle up to its lumpy moon Phobos (above). Or maybe you'd prefer to visit Betelgeuse, a red supergiant star 600 light-years away in the constellation Orion. You can complete both expeditions during your lunch hour with Celestia, a free space-travel simulator created by software engineer Chris Laurel of Seattle, Washington.* The program, which builds on NASA images and astronomical data from sources such as the Hipparcos star catalog, lets you tour the solar system and voyage to more than 100,000 stars. Enthusiasts have crafted hundreds of programs that boost the number of objects you can visit and add more detail to ones already in Celestia—for instance, one offers a high-resolution view of the sun's surface complete with solar flares. Download these supplements at the Celestia Motherlode.† It can take practice to master Celestia's controls, and the program requires a powerful graphics card to display all features.

* www.shatters.net/celestia

† www.celestiamotherlode.net

DATABASE

Tallying America's Health



The number of overweight and obese adults in the United States has ballooned by 20% since the early 1960s, reaching 64%. But the rate of adult obesity has leveled off since the late 1990s (right). From the girth of the nation to the prevalence of asthma, the National Center for Health Statistics's Web site stashes the numbers that reveal Americans' physical and mental well-being.

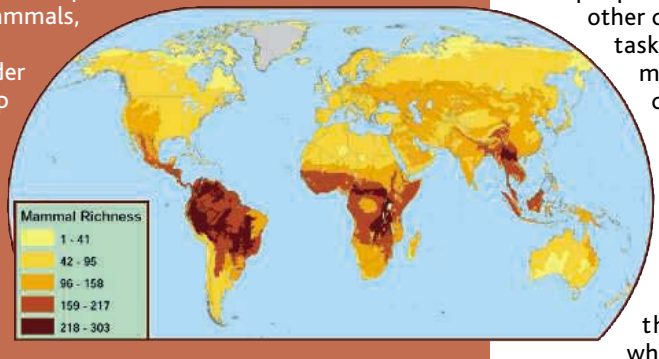
The clearinghouse lets you prowl the Centers for Disease Control and Prevention's (CDC's) data collections. Click on the FASTATS index to track down nuggets of information such as the number of deaths from Alzheimer's disease every year (nearly 59,000) and the incidence of diabetes (6.6% of the adult population). A feature called WONDER guides visitors to a host of CDC documents and databases. For example, users can dig up the number of AIDS cases in different cities and view county-by-county maps of injury-related deaths. You can also read the latest results from reports such as the National Health and Nutrition Examination Survey, which regularly gauges Americans' health.

www.cdc.gov/nchs

TOOLS

A World of Vertebrates

Whether you're mad about the muskox or keen on the kea, a New Zealand parrot, check out WildFinder from the World Wildlife Fund in Washington, D.C. The new database lets users map the distributions of 30,000 species of terrestrial amphibians, reptiles, mammals, and birds.



Searching for a species in WildFinder doesn't return a conventional range map but instead shows which of the world's 825 ecoregions the animal inhabits—areas with similar environments and species. For example, the muskox roams 11 ecoregions, including the northern Canadian shield taiga and the Beringia lowland tundra of Alaska. WildFinder's maps draw on information from field guides, online databases, scientific papers, and other sources. You can also scan the database geographically to retrieve a list of the vertebrates that dwell in a particular city or country. For a global view of species diversity, visit the Map Gallery, whose offerings include this chart of mammal species numbers.

www.worldwildlife.org/wildfinder

DATABASE

When Proteins Get Fat

Bacteria rely on protein-lipid combinations known as lipoproteins to glom onto surfaces, sense their surroundings, slurp up nutrients, shuttle DNA to other cells, and perform other life tasks. Researchers can analyze more than 270 of the molecules at DOLOP, a database from the Medical Research Council Laboratory of Molecular Biology in Cambridge, U.K. Entries describe each protein, indicate its size and function, and provide links to the Swiss-Prot database, where you can parse the molecule's sequence and structural features. The site also explains the synthesis of lipoproteins and describes the lipobox, a characteristic amino acid string to which lipids attach.

www.mrc-lmb.cam.ac.uk/genomes/dolop

Send site suggestions to netwatch@aaas.org. Archive: www.sciencemag.org/netwatch

CREDITS (TOP TO BOTTOM): CHRIS LAUREL; CDC; WWF

This Week

PAGE 681
Transposon makes a fly resistant

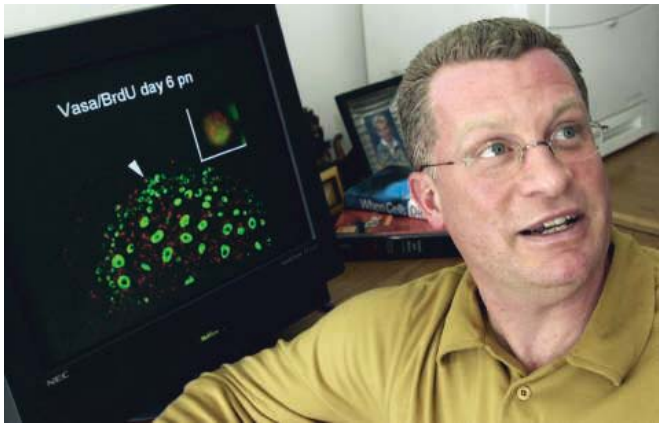
684
Tussle over a gene bank

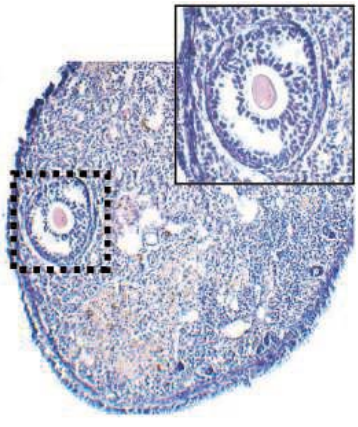
REPRODUCTIVE BIOLOGY

Controversial Study Finds an Unexpected Source of Oocytes

Scientists have made some surprising claims about bone marrow and blood cells in the last few years, but this week brings perhaps the most surprising of all: that cells in the bone marrow and blood are a source of developing oocytes found in the ovaries. If true, this work in mice would rewrite the current understanding of the female reproductive system. It could also open new discussions about the ethics and potential consequences of bone marrow and even blood donation.

Tilly, Johnson, and colleagues have now dropped another bombshell at a meeting* and in the 29 July issue of *Cell*: They report that they have found ovary-replenishing germ cells in the bone marrow and circulating blood of adult mice. They build their case on several lines of evidence. First, looking for the source of oocyte stem cells that might explain their previous results, the team found signs that genes typical of germ cells were expressed in samples of bone marrow from mice and from humans. They also found that the level of at least one of these genes, called *Mvh*, varies during the animals' estrus cycle. That made them wonder if cells in the bone marrow might be a source of new oocytes.





Blood borne? Jonathan Tilly of Massachusetts General Hospital and colleagues claim that bone marrow transplants and blood transfusions can prompt the ovaries of genetically infertile mice to begin producing oocytes (*inset*).

Although the study's authors do not have evidence that such blood-derived oocytes could be fertilized and develop into babies, they suggest that human donors might be sharing germ cells along with their lifesaving immune cells and clotting factors. They also say they hope this work will lead to new treatments for infertility, especially for women who must undergo chemotherapy.

For decades, scientists have thought that female mammals are born with a lifetime supply of potential oocytes in the ovary. That view was challenged last year by Jonathan Tilly, Joshua Johnson, and their colleagues at Massachusetts General Hospital in Boston, who reported in a controversial paper in *Nature* that new oocytes could form throughout an adult mouse's lifetime (*Science*, 12 March 2004, p. 1593). That finding has not been replicated in another lab.

To check that idea, the team treated mice with two chemotherapy drugs that cause infertility, cyclophosphamide and busulfan. Mice that received the drugs, as expected, suffered extensive ovary damage and stopped producing new oocytes. But in the ovaries of treated mice that later received bone marrow transplants from female donors, the scientists found "several hundred" oocyte-containing follicles at various stages of maturity.

The effect of treatment was rapid: New oocytes appeared 28 to 30 hours after a transplant. Some oocyte development experts are dubious, noting that fruit fly oocytes take a week to mature from stem cells. "You just can't do it in a day," says Allan Spradling of the Carnegie Institute of Washington in Baltimore, Maryland. But Tilly says the oocytes might begin to mature in the bone marrow and continue developing as they travel through the bloodstream.

The team also reports using bone marrow and blood transplants to prompt the growth of oocytes in mice that are genetically infertile. Mice with a mutation in a gene called *ataxia-telangiectasia mutated* can't produce mature germ cells, and their ovaries usually lack follicles and developing oocytes. But after receiving either bone marrow or blood from healthy donors, the team reports, the animals' ovaries started producing follicles containing healthy-looking oocytes. The team concludes that bone marrow provides a continuous source of germ cell stem cells to the ovaries throughout adult life.

So far, however, they have not been able to prove that these cells can trigger ovulation or give rise to new offspring. "Until the authors have shown that the putative oocytes are functional, we should be cautious," says Margaret Goodell of Baylor College of Medicine in Houston, Texas, who studies bone marrow stem cells. She and others say the markers the team used to identify oocytes can be misleading. For instance, similar techniques have led others to conclude mistakenly that bone marrow cells had become neurons or lung cells. "It will be important to transplant [green fluorescent protein] positive bone marrow cells into GFP-negative adult mice to test whether those mice go on to give birth to GFP-positive pups," says Sean Morrison of the University of Michigan, Ann Arbor. "This experiment should be straightforward."

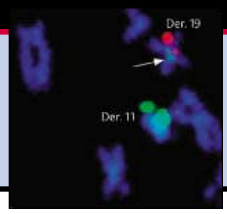
Tilly says the team is working on such experiments but has had to find a new approach because the drugs they were using can damage the uterus and fallopian tubes, possibly preventing mice from becoming pregnant.

Turning to the clinic, Tilly suggests that the mouse results could explain a number of surprising reports of cancer patients and others who were expected to be infertile but who gave birth to children after receiving bone marrow transplants. One patient ▶

* Society for the Study of Reproduction, Quebec City, Canada, 24–27 July.

688
10 years after the spotted owl

691
Academy chief in the spotlight



693
A new genome project

with Fanconi's anemia, for example, had a single menstrual period and then entered menopause at age 12. After receiving a bone marrow transplant from a sibling, Tilly says, her periods resumed, and she later gave birth to two children.

Although genetic tests of patients and their children might answer the question, Tilly says, they would be ethically problematic. And such cases wouldn't necessarily be easy to detect, he says, because bone marrow donors are often siblings.

Even if the new oocytes can't be fertilized, Tilly says, they may nevertheless enhance a woman's fertility. He speculates that they may function as "drone oocytes" that keep the ovary functioning to support the original "queen" oocytes set aside for procreation. If so, he says, the results open new possibilities for preserving or restoring the fertility of young cancer patients and might even provide a way to postpone menopause.

But until the team produces mice that can be traced without a doubt to a bone marrow donor, scientists are likely to remain wary. "The experiments will have a stimulating effect on the field," says Hans Schöler of the Max Planck Institute for Molecular Biomedicine in Münster, Germany, "even if they stir quite some controversy." **–GRETCHEN VOGEL**

PALEONTOLOGY

Dinosaur Embryos Hint at Evolution of Giants

Paleontologists have long assumed that giant dinosaurs called sauropods, like all other dinosaurs, evolved from smallish bipedal ancestors and dropped down on all fours only as their bodies grew too large to be carried on two feet. But when they examined a pair of embryos dug up about 30 years ago—the oldest fossilized dinosaur embryos so far discovered—they got a surprise. As described on page 761 by Robert Reisz of the University of Toronto's Mississauga campus in Canada and colleagues, the embryos suggest that sauropods were already quadrupedal even as smaller creatures. "This would be significant because it means we might have to re-evaluate the origin of many features in sauropod skeletons we assumed had to do with weight support," says Matthew Bonnan of Western Illinois University in Macomb.

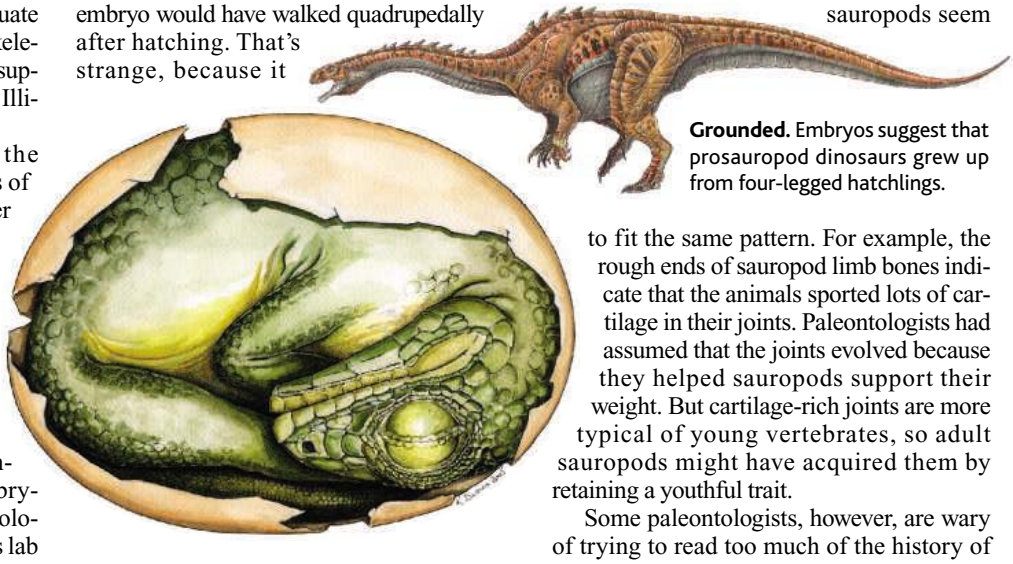
The clues are indirect, because the embryos are not sauropods but members of their closest kin, a group of much smaller herbivores called the prosauropods. Paleontologists found them inside remarkably well-preserved eggs of a 5-meter-long animal called *Massosponodylus*, which 190 million years ago roamed the floodplains of what is now South Africa. "It's a really cool discovery," says Kristi Curry Rogers of the Science Museum of Minnesota. The eggs clearly contained embryonic bones, but only recently did paleontologists dare to prepare them. It took Reisz's lab technician Diane Scott more than a year of full-time work to expose the delicate bones of the 6-centimeter-long eggs. As Reisz studied the specimens with colleagues from the Smithsonian Institution and the University of the Witwatersrand in Johannesburg, South Africa, he identified the largish skull as that of *Massospondylus*.

What was unusual was the rest of the body. "The proportions are just ridiculous," Reisz says. The neck was long, the tail short, and the hind and forelimbs were all roughly the same length. "It was an awkward little animal," he concludes. Because of the lack of developed teeth, huge head, and tiny pelvis (where leg muscles attach), the group proposes that *Massospondylus* hatchlings would have required parental care. "This is certainly suggestive but very difficult to test," says Martin Sander of the University of Bonn, Germany.

To Reisz, the horizontal neck, heavy head, and limb proportions all suggest that the embryo would have walked quadrupedally after hatching. That's strange, because it means that as the *Massospondylus* hatchlings developed, they had to become bipedal—a pattern of development almost unheard of among vertebrates. To figure out how the hatchlings changed as they matured, the researchers measured nine other *Massospondylus* fossils of various sizes. They found that the neck grew much more rapidly, relative to the femur, than the rest of the body did, while the forelimbs and skull grew more slowly.

If the earliest sauropods also developed from embryos with quadrupedal proportions, Reisz and his colleagues propose, sauropods may have become quadrupedal adults by retaining their juvenile state into adulthood, a phenomenon called pedomorphosis. "It sheds some light in the evolutionary pathways through which the peculiar adaptations of giant dinosaurs were attained," says Eric Buffetaut of France's major basic research agency, CNRS, in Paris.

Bonnan notes that other traits of adult sauropods seem to fit the same pattern. For example, the rough ends of sauropod limb bones indicate that the animals sported lots of cartilage in their joints. Paleontologists had assumed that the joints evolved because they helped sauropods support their weight. But cartilage-rich joints are more typical of young vertebrates, so adult sauropods might have acquired them by retaining a youthful trait.



Grounded. Embryos suggest that prosauropod dinosaurs grew up from four-legged hatchlings.

Some paleontologists, however, are wary of trying to read too much of the history of sauropod evolution from two embryos. So little is known about dinosaur embryology, they say, that it's dicey to reconstruct the locomotion of hatchlings and extrapolate to other taxonomic groups. "It's a stunning find," says Anusuya Chinsamy-Turan of the University of Cape Town, South Africa, but "I have all these questions." **–ERIK STOKSTAD**

ILLUSTRATIONS (TOP TO BOTTOM): GABRIEL LIO/COURTESY OF UNIVERSITY OF TORONTO AT MISSISSAUGA; KEVIN DUPUIS

The highest purity research products in the world… all from a unique company in New England.

UNCOMPROMISED PURITY

In today's world, the molecular biology industry demands nothing less than the very best that science has to offer. There is no room for compromise.

At New England Biolabs we understand this fact. For over 30 years, we have led the industry in the discovery and production of enzymes for molecular biology applications. And through our extensive efforts in the cloning and overexpression of restriction/modification systems, we have set the standards for quality and price.

Benefit from our uncommon philosophy of doing business where impressive production efficiencies are passed along in the form of lower prices and improved purity.

UNSURPASSED EXPERTISE

Discover the world's largest collection of recombinant as well as native enzymes for DNA technology, all backed by unsurpassed scientific expertise.

And, if you want to learn more about our products, technical service or extensive distribution network, visit www.neb.com or contact us at info@neb.com or 1-800-NEB-LABS.

New England Biolabs, Inc. – celebrating 30 years as a world leader in the production and supply of reagents for the life science industry.

NEB has relocated its headquarters to Ipswich, MA. Our new campus includes a state of the art 140,000 sq. ft. research and production laboratory and a beautifully restored Victorian mansion housing our administrative offices.

PRODUCTS YOU TRUST. TECHNICAL INFORMATION YOU NEED. **www.neb.com**

- **New England Biolabs Inc.** 240 County Road, Ipswich, MA 01938 USA 1-800-NEB-LABS Tel. (978) 927-5054 Fax (978) 921-1350 info@neb.com
- **Canada** Tel. (800) 387-1095 info@ca.neb.com
- **Germany** Tel. 0800/246 5227 info@de.neb.com
- **UK** Tel. (0800) 318486 info@uk.neb.com
- **China** Tel. 010-82378266 beijing@neb-china.com

NEW ENGLAND BioLabs® Inc.
the leader in enzyme technology

DISTRIBUTORS: Argentina (11) 4372 9045; Australia (07) 5594-0299; Belgium (0800)1 9815; Brazil (11) 3622 2320; Czech Rep. 0800 124683; Denmark (39) 56 20 00; Finland (09) 584-121; France (01) 34 60 24 24; Greece (010) 5226547; Hong Kong 2649-9988; India (044) 220 0066; Israel (3) 9021330; Italy (02) 381951; Japan (03) 3272-0676; Korea (02) 556-0311; Malaysia 603-80703101; Mexico 52 5525 5725; Netherlands (033) 495 00 94; Norway 23 17 60 00; Singapore 67775366; Spain 902.20.30.70; Sweden (08) 30 60 10; Switzerland (061) 486 80 80; Taiwan (02) 28802913

EVOLUTION

Rogue Fruit Fly DNA Offers Protection From Insecticides

Genomes are full of DNA that doesn't belong there. Called transposons, these small bits of sequence jump between chromosomes, often disrupting genes in the process. But sometimes, these interlopers do some good. Dmitri Petrov, a population geneticist at Stanford University in California, and his colleagues have discovered a transposon that, by changing a gene, seems to help fruit flies evolve resistance to certain insecticides. The work, reported on page 764 of this issue of *Science*, is one of a growing number of examples of natural selection preserving transposons, indicating that "they may play a much larger role in evolutionary novelty than is currently appreciated," says Todd Schlenke, an evolutionary geneticist at Cornell University.

A little help from ... Although transposable elements tend to be harmful, one has helped make *Drosophila melanogaster* tougher to kill.

CREDIT: DENNIS KUNKEL/VISUALS UNLIMITED

Typically, researchers have stumbled on such beneficial transposons while searching for mutations involved in disease or traits such as resistance to toxins. The general assumption has been that these movable DNA elements have long been intertwined with the gene in question. But Petrov and his colleagues demonstrated that transposon-mediated evolution can happen in real time to create novel solutions to changing conditions.

Working with Petrov, Stanford graduate student Yael Aminetzach had determined which of the 16 members of the *Doc* family of transposable elements were common in populations of the fruit fly *Drosophila melanogaster*. One stood out, *Doc1420*. Unlike other *Doc* transposons, which proved to be quite rare, this one appeared in 80% of fruit flies tested from eight different countries, suggesting that it plays some useful role. "The paper is a tour de force of population genetics," says David Heckel, a geneticist at the Max Planck Institute for Chemical Ecology in Jena, Germany.

When the Stanford researchers then looked more closely at this transposon, they found that it had landed in a gene that, to date, has defied characterization. The gene exists intact in distantly related fruit flies, suggesting that it has a key function—one that was disrupted as *Doc* elements jumped around the *D. melanogaster* genome. By comparing *Doc1420* to the other *Doc* sequences, Aminetzach and graduate student Michael MacPherson estimate that *Doc1420* buried itself in this gene 90,000 years ago but did not become widespread until between 25 and 240 years ago, when human activities began to alter the environment dramatically. This recent expansion suggested that, rather than rendering the gene nonfunctional, the transposon altered it, possibly resulting in a different protein product—one that became important to the species' survival.

The sequence of the unaltered gene provided a clue to this new gene's role. That sequence resembles that of genes for choline metabolism, which operate in nerves affected by organophosphate pesticides. To test whether the new protein was involved in this pathway, the researchers bred fruit flies to create strains that differed only in whether they carried the *Doc1420* insertion. The *Doc1420* strain fared much better when Aminetzach and her colleagues treated the insects with an organophosphate insecticide: 19% died, compared to 68% of the fruit flies lacking *Doc1420*.

Researchers have already identified a few other examples of transposon-induced insecticide resistance, but this is the first to disrupt a gene whose protein is not a target of the pesticide, Petrov says. But Schlenke, Heckel, and others say that more work is needed to verify the transposon's role in resistance. "The data showing pesticide resistance [are] very weak," notes Richard ffrench-Constant, a molecular entomologist at the University of Bath, U.K.

Nonetheless, Martin Feder of the University of Chicago is quite enthusiastic. "The paper is the latest in a series of recent discoveries that transposons can play a role in 'real time' microevolution in natural populations," he says. "The phenomenon is [now] difficult to ignore."

–ELIZABETH PENNISI

ScienceScope

Wilmut Seeks Fresh Eggs

Cloning researcher Ian Wilmut of the University of Edinburgh and his colleagues are asking for permission from a national oversight board in the U.K. to use freshly donated human oocytes from volunteers in their attempts to create stem cells through nuclear transfer. South Korean research has suggested that it's much more efficient to create cloned embryos from the oocytes of healthy young donors than those left over from fertility treatments (*Science*, 17 June, p. 1777). Oocyte donation can lead to serious medical complications, but Wilmut's colleague Christopher Shaw of King's College London says the group has already been approached by several potential donors. The Human Fertilisation and Embryology Authority must approve the donations.

–GRETCHEN VOGEL

Nuke Reprocessing Inches Ahead

U.S. negotiators reportedly agreed earlier this month to drop a key demand that was blocking a treaty with Russia to reprocess 34 metric tons of weapons-grade plutonium in both nations. The United States had wanted to protect contractors making the fuel suitable for Russian power plants from lawsuits, a provision found in a 1992 nonproliferation agreement. "We've essentially lost 2 years of time," said a spokesperson for the nonprofit Russian American Nuclear Security Advisory Council in Washington, D.C., which had opposed the immunity clause. The agreement, which has not been finalized, must be approved by the Duma, although under U.S. law it does not require congressional approval.

–ELI KINTISCH

Help for Russian Science

Following months of closed-door negotiations, the Russian government and scientific community leaders have struck a compromise to restructure the underfunded Russian Academy of Sciences (RAS) and streamline federal research. For their part, the academicians have agreed to discuss a concept that initially proposed reducing the number of RAS institutions from more than 450 to between 100 and 200. In turn, the government has reportedly agreed to raise researchers' monthly salaries, currently between $100 and $200, to about $1050 by 2010. This fall, a Duma committee will try to hammer out details.

–ANDREY ALLAKHVERDOV AND VLADIMIR POKROVSKY

NATIONAL SCIENCE FOUNDATION

Two Mines in Running for Underground Lab

The U.S. National Science Foundation (NSF) has decided that it's in the business of experimentation, not excavation. On 21 July, the $5.5 billion research agency chose two established mines—the Homestake Mine in Lead, South Dakota, and the Henderson Mine in Empire, Colorado—as possible sites for a multipurpose underground laboratory. In doing so, NSF passed over four "green field" sites that would have required builders to excavate thousands of feet of rock and existing sites in Nevada and Ontario, Canada.

The proposed Deep Underground Science and Engineering Laboratory would house experiments in particle physics, geoscience, and microbiology. The original idea was for federal lawmakers to salvage Homestake for scientific ends before it was abandoned and flooded (*Science*, 6 June 2003, p. 1486). But that initiative was derailed by political and environmental considerations, leaving NSF free to pursue a more deliberate process that engaged a larger section of the scientific community. Last October, the agency solicited proposals for other sites.

The two preliminary winners in that competition "stood out significantly above the rest" because they are deep, have desirable geologic characteristics, and come with some infrastructure already in place, says John Lightbody, executive officer of NSF's division of physics. Each team will receive $500,000 to work up a full conceptual design for the laboratory, which backers hope could win funding as early as 2009.

Both mines present challenges. Henderson is an active molybdenum mine, meaning that researchers would have to coordinate their activities with the mining operations. But a working mine also provides functioning lifts, vents, and other infrastructure that researchers can take advantage of, says Chang Kee Jung, a particle physicist at Stony Brook University in New York and spokesperson for the Henderson Mine collaboration.

Rocky Mountain high. The Henderson molybdenum mine west of Denver, Colorado, has made the first cut to become an underground laboratory.

In contrast, the abandoned Homestake gold mine was sealed in 2003 and is currently filling with groundwater. Once it reaches 1480 meters below the surface, possibly by 2007 or 2008, the mine's infrastructure could be ruined. However, South Dakota officials plan to open the upper levels of the mine for experiments and begin pumping out water as early as 2006, says Dave Snyder, executive director of the South Dakota Science and Technology Authority. Barrick Gold Corp. has agreed to transfer the mine to the state if the state legislature approves funds to open the site or if NSF builds the lab at Homestake, Snyder says.

Last weekend, the University of Minnesota, Twin Cities (UMTC), hosted a workshop to discuss the scientific mission of an underground lab. Some scientists feel that NSF short-circuited its own process by narrowing the choices to just two alternatives and excluding green-field sites. "If what they wanted was cheap and deep, they could have told us that right away, and we wouldn't have had to do all this work," says Priscilla Cushman, a UMTC physicist who worked on a losing proposal to dig the laboratory at the Soudan Mine in Minnesota.

Despite their disappointment, most scientists are expected to rally behind one of the two remaining collaborations, says Bernard Sadoulet, a cosmologist at the University of California, Berkeley. "I'm convinced that the science is so compelling that the community will pull together," says Sadoulet, who is leading a study to define the scientific mission of the lab. That teamwork, however, is only the first step in a long process. **–ADRIAN CHO**

BIODEFENSE

U.S. University Backs Out of Biolab Bid

The University of Washington (UW), Seattle, last week abruptly abandoned its attempt to build a biosafety level 3 (BSL-3) facility to study infectious diseases and bioterrorism agents. University officials say they were unable to come up with the $35 million required by the National Institutes of Health (NIH) to keep the proposal alive. But there was also intense opposition to the proposed $60 million facility from community activists, who saw it as a public health and safety hazard.

The university was one of several institutions that applied last December for a Regional Biocontainment Laboratory grant, part of a post–9/11 push to increase the nation's ability to study infectious agents. NIH has set aside approximately $125 million for a second national competition to complement an earlier round of nine labs funded in 2003 (*Science*, 10 October 2003, p. 206). It expects to make from five to eight awards for the BSL-2 and -3 labs, which handle materials such as plague.

Three public forums in Seattle this spring drew hundreds opposed to the 5200-square-meter facility, which would have employed 100 scientists and staff. In May, university officials noted that community trust "has been dramatically undermined" and that building the lab despite opposition could prove "devastating" to community relations. An NIH grant to Boston University to build a lab to study even more dangerous biological agents is moving ahead despite citizen protests (*Science*, 28 January, p. 501).

Despite that opposition, chief UW spokesperson Norm Arkans says that the real deal breaker for Washington was money: "We knew it would be difficult to raise the $35 million, since the university has a number of capital needs." A letter from NIH asking for details of its cost-sharing plans triggered the university's pullout, according to Arkans. NIH officials declined comment on the competition, the winners of which are expected to be announced in September.

Community activists were delighted, but they don't take credit for preventing construction. "I think it came down to money," says Kent Wills, head of the University Park Community Club. And some scientists are unhappy with the university's withdrawal. "We desperately need better facilities in the Pacific Northwest," says Samuel Miller, a UW infectious disease specialist. The decision won't keep BSL-3 work away from Seattle: Two dozen university labs already provide that level of containment.

–ANDREW LAWLER

CREDIT: HENDERSON DUSEL

TISSUE ENGINEERING

Technique Uses Body as 'Bioreactor' to Grow New Bone

Tissue engineers have long dreamed of starting with a small clutch of cells in a petri dish and growing new organs that can then be transplanted into patients. The strategy has worked for relatively simple, thin tissues such as skin and cartilage that don't depend on a well-formed network of blood vessels to deliver food and oxygen. But it hasn't panned out for more complex tissues shot through with vessels, such as bone and liver. Now a novel approach to tissue engineering that grows bone inside a patient's own body could change all that.

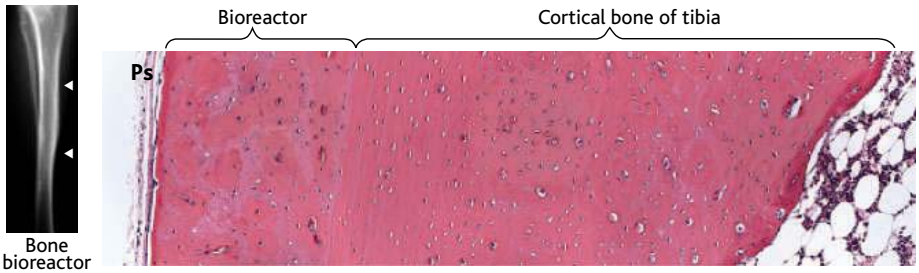
In a paper published online this week by the *Proceedings of the National Academy of Sciences*, researchers from the United States, the United Kingdom, and Switzerland report that they grew large amounts of new bone alongside the long leg bones of rabbits. When they harvested and transplanted the new bone into bone defects in the same animal, the defects healed and were indistinguishable from the original.

"This is a fresh, new strategy for tissue engineering that relies on the body's own capacity to regenerate itself," says Antonios Mikos, a tissue engineering specialist at Rice University in Houston, Texas. "I think it will have an enormous impact on the field."

The field of tissue engineering could use some help. Attempts to grow complex tissues outside the body have progressed in fits and starts. Italian researchers, for example, have coaxed bone marrow cells injected into a ceramic matrix to create new bone. But organisms have been unable to resorb and remodel the tissue, as occurs with normal bone. To avoid such problems, researchers led by tissue engineers Prasad Shastri at Vanderbilt University in Nashville, Tennessee, and Molly Stevens and Robert Langer at the Massachusetts Institute of Technology in Cambridge decided to see if they could let the body handle it itself.

Bones are sheathed in a thin membrane of cells called the periosteum. If a small wound or fracture occurs, cells in the periosteum can divide and differentiate into replacement tissue, including new bone, cartilage, and ligaments. Shastri wanted to see if he and his colleagues could use this same wound-healing response to generate new tissue.

The researchers injected a surgical saline solution between the tibia—the long, lower leg bone—and the periosteum of white rabbits, a standard small animal model for studying bone. This created a small, fluid-filled cavity into which they hoped new bone would grow. To prevent the cavity from collapsing as the saline is absorbed by the body, the researchers injected a gel containing a calcium-rich compound called alginate. Previous studies have suggested that calcium helps trigger cells in the periosteum to differentiate into new bone, and that is exactly what happened, the researchers report. Within a few weeks, the alginate cavities were filled with new bone. And when that bone was removed and transplanted to damaged bone sites within the same animals, the new bone integrated seamlessly.



Good as new. A surgically formed cavity acts as a "bioreactor" to grow new bone between the periosteum (Ps) and mature bone in the tibia of a rabbit (*above*), producing a slight bulge of new bone (*left*).

"I think the strength of this approach is its simplicity," Mikos says. "It doesn't rely on the delivery of exogenous growth factors or cells." That could make it a boon to orthopedic surgeons, who often need to harvest large amounts of bone from patients to fuse vertebrae in spinal fusions. That harvested bone usually comes from a patient's hip, a procedure that often produces pain for years. But if this approach works in people, it could enable physicians to generate new bone alongside a patient's shin, for example, which could then be transplanted to other sites.

The technique could also prove useful for other tissues. With a few tweaks, says Shastri, it works to generate healthy new cartilage. Now the team is looking to see if it can be used to generate liver tissue as well. If so, it may turn tissue engineers' dreams into reality.

–ROBERT F. SERVICE

CREDITS (LEFT TO RIGHT): M. M. STEVENS *ET AL. PNAS* (2005); NPO LAVOCHKIN/THE PLANETARY SOCIETY

ScienceScope

Deadly Bacteria in China

A mysterious disease that has caused at least 19 deaths in China's Sichuan Province is being blamed on *Streptococcus suis* type 2, a bacteria common in pigs throughout the world. Robert Dietz, a spokesperson for the World Health Organization in Manila, says laboratory confirmation is still pending but that the reported symptoms seem to be consistent with human *S. suis* infection. Human cases are rare, Dietz says, making it surprising that China has so suddenly recorded 67 to date. Although a more virulent strain of the bacterium could be the culprit, Dietz thinks that China's "enhanced surveillance capabilities" are a more likely explanation. But Marcelo Gottschalk, a *S. suis* expert at the University of Montreal in Canada, doubts the diagnosis. "It's just very strange for so many people to be infected in such a short time," says Gottschalk, who notes that hearing loss—a common human *S. suis* symptom—has not been reported in Sichuan.

–DENNIS NORMILE AND MARTIN ENSERINK

Updates

■ An epidemiologist who was subpoenaed for 25 years' worth of his data on lead exposure and health effects in children has won a compromise with paint companies (*Science*, 15 July, p. 362). Attorneys for the University of Cincinnati have agreed that Kim Dietrich will release a small subset of his data on children's IQs and lead levels that was recently published as part of a pooled analysis. The companies say they need the data to defend themselves against a lawsuit filed by the state of Rhode Island.

■ White House Office of Science and Technology Policy officials Kathie Olsen and William Alan Jeffrey were confirmed by the Senate last week for new positions as deputy National Science Foundation director and head of the National Institute of Standards and Technology, respectively. Olsen is a 52-year-old neuroscientist with experience at NASA; Jeffrey, 45, served previously at the Defense Advanced Research Projects Agency.

■ The Russian review board investigating the failed June launch of Cosmos 1, a privately funded solar sail spacecraft, has concluded that it never reached orbit due to a pump failure.

VETERANS AFFAIRS

Gene Bank Proposal Draws Support—and a Competitor

The U.S. Department of Veterans Affairs (VA) is quietly moving forward with plans for a national gene bank that would link DNA donated by up to 7 million veterans and their family members with anonymous medical records. The bank, which is widely supported inside and outside the VA, would represent the first massive U.S. gene banking effort. But it is causing a furor among scientists, some VA employees, and politicians from New York state. They charge that top VA officials accepted a gene bank proposal from a cancer biologist at Stratton VA Medical Center and the State University of New York (SUNY), Albany, but are now privately circulating another gene bank plan that may leave Albany out. Most senior officials and scientists involved in both plans declined to comment for this story.

Although some smaller gene banks are sprouting in the United States, none can match those gearing up in Iceland, Estonia, the United Kingdom, and Japan (*Science*, 8 November 2002, p. 1158). In these cases, DNA samples from hundreds of thousands of people are linked with health information stripped of identifiers, making the banks powerful tools for sorting out "the complex interactions between gene and environment that lead to disease," says Alan Guttmacher, deputy director of the National Human Genome Research Institute (NHGRI) in Bethesda, Maryland.

The VA, say outside scientists, is a natural home for such a project because health records for the 7 million people it serves are computerized and standardized. The VA "has not only samples but histories," says Karen Hitchcock, president of SUNY Albany until early 2004 and now the principal and vice chancellor at Queens University in Kingston, Canada. Although there are potential disadvantages to a VA bank—namely low numbers of females, if veterans but not family members are included—the population includes minorities underrepresented in gene banks overseas, says Guttmacher.

Not in the bank. Paulette McCormick's proposal seemed to have won approval, but the VA is now circulating a similar plan of its own.

According to documents obtained by *Science*, in July 2002, Paulette McCormick, who held joint appointments at the Stratton VA Medical Center and as head of SUNY Albany's Center for Functional Genomics, sent a gene bank proposal to Mindy Aisen, then the VA's deputy chief of research and development and now chief of the VA's rehabilitation research division. McCormick's plan was to collect blood samples from at least 2 million volunteers. The data bank would be open "to VA scientists and other academic and industry scientists" after their projects were approved by the VA and the bank's scientific and ethics committees, one version of her proposal states. The samples would be owned by the VA; they and computers containing the data were to be stored in locked rooms at SUNY Albany. McCormick also proposed having companies pay to access gene bank data as a means of funding the bank. Strict privacy controls would protect DNA donors.

SUNY Albany officials and New York politicians saw the plan as a flagship project that could raise the profile of the university and the state. "We all kind of whooped. It was an absolutely fantastic idea," says Hitchcock.

On 11 December 2003, the VA signed an agreement with Albany suggesting that it would move forward with McCormick's ▶

AVIAN INFLUENZA

WHO Faults China for Lax Outbreak Response

Worried that Asia's bird flu outbreak could be on the verge of spreading worldwide, increasing the risk of a human pandemic, international health organizations are warning that China is not rigorously following up on a recent outbreak of the deadly H5N1 strain among wild birds in the western Qinghai region. In particular, the World Health Organization (WHO) is pressing Chinese officials to study migratory birds to see whether they may be able to spread the virus to previously unaffected areas. Chinese scientists point out that they have already sequenced virus from migratory birds and made the results publicly available through GenBank.

Concerns are focused on the H5N1 outbreak at China's Lake Qinghai. The unprecedented 6000 death toll among wild birds, previously only slightly affected by infections, has experts worried that the virus has become more lethal and that surviving migratory birds could carry it to wintering grounds in India, which has not yet reported any H5N1 outbreaks.

To assess this risk, WHO and the United Nations Food and Agriculture Organization (FAO) have urged Chinese authorities to sample surviving birds to see whether any are carrying the virus without obvious symptoms, as well as to tag birds for tracking. China's Ministry of Agriculture could not be reached for comment. But in an interview with the *Wall Street Journal* that appeared on 19 July, Jia Youling, director general of the ministry's Veterinary Bureau, was quoted as saying they haven't tested live migratory birds "because in catching them, it is easy to harm them." FAO animal epidemiologist Juan Lubroth in Rome says that there are humane ways of testing of live birds. Such data, he adds, "would allow for preventive actions on the ground, such as vaccinating domestic poultry flocks near known rest areas" along migratory routes.

Roy Wadia, a spokesperson for WHO in Beijing, says China has also not yet responded to requests for isolates of the virus circulating in Qinghai. Time is of the essence, he says, because authorities want to determine whether the virus has changed before the return migration. Wadia was unaware that DNA sequence information from samples from Lake Qinghai had been deposited in GenBank by a group at China's Institute of Microbiology; they reported online in *Science* that the virus appears to have changed in ways that could make it more lethal (*Science*, 8 July, p. 231).

Meanwhile, Indonesia confirmed its first human deaths from bird flu, among a family that apparently had no contact with infected poultry—the usual route of transmission—raising questions about possible human-to-human transmission. And as *Science* went to press, Russian officials were trying to determine the H5 subtype responsible for an outbreak of avian influenza among poultry in Novosibirsk.

—DENNIS NORMILE

CREDIT: COURTESY OF THE UNIVERSITY AT ALBANY

plan and base the bank in New York state. SUNY Albany modified plans for a cancer research center then under construction, making "add-ons" to accommodate space for a gene bank at a cost of "multiple millions," says Hitchcock. In an e-mail sent on 19 March 2004, Jonathan Perlin, now VA undersecretary for health, wrote to three colleagues in VA headquarters that the gene bank "is a VA resource, first and foremost, and Albany would be a lead partner."

That May, a small VA delegation, including Perlin, traveled to Albany and met with New York State Senator and majority leader Joseph Bruno (R) and New York Governor George Pataki (R), say sources familiar with the meetings. At the time, it was generally understood that New York would supply most of the project's pilot funding—estimated at $10 million—while the VA would offer nominal support, such as staff to collect blood samples.

But behind the scenes, the project was unraveling. An e-mail from Perlin sent in February 2004 noted that McCormick's proposal "has raised significant ethical, privacy and operational issues." An e-mail from Nora Egan, then VA Secretary Anthony Principi's chief of staff, reported that the secretary felt that "issues related to medical ethics, privacy, … and benefit to be derived by VA" needed to be addressed. Precise concerns were not specified. A fall 2003 review of McCormick's proposal by the director of the VA's National Center for Ethics in Health Care had concluded: "On the whole, the … Gene Bank proposes ethically appropriate measures to protect subjects' privacy and the confidentiality of their personal health and genetic information."

Earlier this year, VA officials at the agency's headquarters began circulating memos of a separate gene bank proposal, reportedly crafted by Perlin, Timothy O'Leary, who heads VA's Biomedical Laboratory Research and Development Service, and Stephan Fihn, acting head of VA research and development until 31 May 2005. A recent confidential draft, obtained by *Science*, is dated 13 July 2005.

Conceptually, the proposal is similar to McCormick's: It recommends gathering blood samples from "all enrollees" in the VA system over 5 years and linking them "to data in other clinical and administrative databases" within the VA. Clinical information would be stored in "highly secure" areas. A scientific advisory committee would offer advice on specimen collection, storage, and other matters; the proposal notes that NHGRI Director Francis Collins has agreed to serve on this committee. (Collins declined to comment.) Biotechnology firms seeking access to the gene bank for specific projects could provide "commercial support." Initial costs are pegged at $40 million to $60 million, and the proposal notes that given tight federal budgets, Congress is unlikely to supply the funds. The proposal diverges from McCormick's in its suggestion that the bank's infrastructure be based in Texas or in Colorado, the home of VA Secretary James Nicholson, to "capitalize on VA support" in those states.

"In my view, there's an evolution in thinking rather than a competition," says Fihn, who explains that on this project of unprecedented scope, VA headquarters realized it had to be in control. Furthermore, Fihn says, it's ludicrous to argue that Albany owned the concept. "Anybody who takes credit for the idea of creating a gene bank in this day and age—it's like saying you invented the Internet," he notes. He can't say what role, if any, Albany will play in the bank and anticipates a competition for participation.

"We were rather upset" by how VA has handled the project, says Richard Roberts, a board member at SUNY Albany's Center for Functional Genomics and the chief scientific officer of New England BioLabs in Ipswich, Massachusetts. Roberts, a Nobel laureate, says it appears that McCormick's idea is being "seized" by "people in Washington."

Last year, as concerns from New York politicians intensified that the VA was backing out of the December 2003 agreement it had signed with SUNY Albany, VA officials asked the agency's general counsel, Tim McClain, for advice. He prepared a memorandum arguing that the agreement isn't binding. "Execution of the subject Agreement by VA did not constitute acceptance of the gene bank research proposal," it reads.

McCormick, meanwhile, has returned full-time to SUNY Albany after being released from the VA last year. Late last month, McCormick's successor on the gene bank, her SUNY Albany colleague Richard Cunningham, was also released from his part-time appointment at the VA, although he continues to work there without pay. "Employee privacy" rules preclude elaborating on those releases, says Linda Blumenstock, a spokesperson for the Stratton VA Medical Center.

–JENNIFER COUZIN

COSMIC-RAY PHYSICS

New Array Takes Measure of Energy Dispute

Amid the incessant hail of cosmic rays striking Earth's atmosphere from outer space, every now and then one comes screaming in with the energy of a walnut-sized hailstone (*Science*, 21 June 2002, p. 2134). Such ultra-high-energy cosmic rays could herald bizarre astronomical phenomena or new fundamental particles, so physicists are eager to know how often they come along. In recent years, Japanese experiments have indicated that the particles are unexpectedly common; American experiments say they're rare. Now the first results from the Pierre Auger Observatory, a gargantuan cosmic ray detector under construction on an ancient lakebed near Malargüe, Argentina, may have pinpointed the crux of the dispute: The apparent energy of the cosmic rays depends on which method is used to measure it.

CREDIT: PIERRE AUGER OBSERVATORY

Cosmic dissonance. Auger's particle detectors (foreground) and telescopes measure different energies for particles from space.

Auger's preliminary findings "go a long way to resolving the difference between the two [previous] data sets," says Floyd Stecker, a theoretical astrophysicist at NASA's Goddard Space Flight Center in Greenbelt, Maryland. Auger researchers will present their results next week at a conference in Pune, India.*

When a high-energy cosmic ray crashes into the atmosphere, it triggers an avalanche of billions of lower energy particles known as an "air shower." Between 1990 and 2004, researchers working with the now-defunct Akeno Giant Air Shower Array (AGASA) about 120 kilometers west of Toyko, Japan, caught some of the particles with detectors on the ground. They compared their readings with the results of a computer simulation to deduce the energy of the original cosmic ray. The researchers spotted about a dozen cosmic rays with energies exceeding 100 exa–electron volts (100 EeV, or 10^{20} eV).

As they stream earthward, the particles ▶

* 29th International Cosmic Ray Conference, 3–10 August.

PIERCE

Grasp the Proteome™

Protein Staining

Imperial™ Protein Stain

A fast, sensitive and robust Coomassie protein stain; 20 minutes to detect 6-12 ng of protein/band

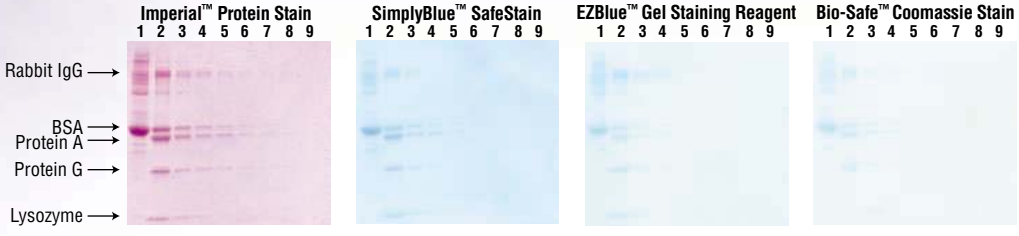
Imperial™ Protein Stain is a ready-to-use Coomassie stain for detecting protein bands in SDS-PAGE and 2-D gels. The stain is a unique formulation of Coomassie R-250 that delivers substantial improvements in protein-staining performance compared to homemade or other commercially available stains.

Highlights:

- **Fast** – detects as little as 6 ng protein/band in just 20 minutes
- **Sensitive** – detects as little as 3 ng protein/band and less with the enhanced protocol
- **Compatible** – with mass spec analysis and protein sequencing
- **Easy-to-use** – no methanol/acetic acid fixation and destaining required

Ordering Information

Product #	Description	Pkg. Size
24615	Imperial™ Protein Stain *Sufficient reagent to stain up to 50 mini gels*	1 L
24617	Imperial™ Protein Stain *Sufficient reagent to stain up to 150 mini gels*	3 x 1 L



Imperial™ Protein Stain is fast and sensitive. Proteins were separated on Novex 4-20% Tris-glycine gels, stained for 5 minutes and destained 3 x 5 minutes in water. **Lane 1:** BSA only (6 µg), Lanes 2-9 contained the indicated proteins at the following concentrations: **Lane 2:** 1,000 ng, **Lane 3:** 200 ng, **Lane 4:** 100 ng, **Lane 5:** 50 ng, **Lane 6:** 25 ng, **Lane 7:** 12 ng, **Lane 8:** 6 ng and **Lane 9:** 3 ng.

www.piercenet.com/impst22e

PIERCE

Tel: 815-968-0747 or 800-874-3723 • Fax: 815-968-7316 • Customer Assistance E-mail: CS@piercenet.com

Outside the United States, visit our web site or call 815-968-0747 to locate your local Perbio Science branch office (below) or distributor

Belgium & Dist.:	China:	France:	Germany:	Hong Kong:	The Netherlands:	United Kingdom:	Switzerland:
Tel +32 53 85 7184	Tel +86 10 8049 9033	Tel 0800 50 82 15	Tel 0228 9125650	Tel 852 2753 0686	Tel 076 50 31 880	Tel 0800 252185	Tel 0800 56 31 40
euromarketing@perbio.com	support@perbio.com.cn	euromarketing@perbio.com	de.info@perbio.com	SalesHK@perbio.com	euromarketing@perbio.com	uk.info@perbio.com	euromarketing@perbio.com

PERBIO

in a shower excite nitrogen molecules in the air and cause them to fluoresce. Researchers working with the High-Resolution Fly's Eye (HiRes) detector at the U.S. Army's Dugway Proving Grounds in Utah use specialized telescopes to detect that light and estimate the energy of the original cosmic ray somewhat more directly. They observed only a few cosmic rays with energies above 100 EeV.

The Auger Observatory possesses both types of detectors. Auger researchers observed dozens of cosmic rays with both the telescopes and the ground detectors and used the "hybrid events" to calibrate the ground detectors without resorting to the computer simulations. The results suggested that the computer simulations overestimate the energies of the cosmic rays by about 25%, says James Cronin, a physicist at the University of Chicago and co-founder of the Auger collaboration.

Some physicists, however, question whether the energy estimates from the fluorescence detectors are really more accurate than those from the simulations. "The Auger measurement clearly explains the difference between the AGASA and HiRes results," says Masahiro Teshima, a cosmic ray physicist at the Max Planck Institute for Physics in Munich, Germany, and former spokesperson for AGASA. "But at the moment, I don't know which is right."

All agree that as it gobbles up data, the massive Auger Observatory should settle the issue once and for all. "In a year and a half with a quarter of the array, we've matched the data set of the existing experiments," Cronin says. "It's looking good." The complete array will comprise 24 light telescopes and 1600 surface detectors covering 300 square kilometers. Within 2 years, Auger researchers expect to have collected seven times more data. **–ADRIAN CHO**

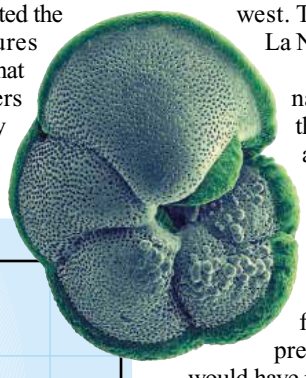
CLIMATE CHANGE

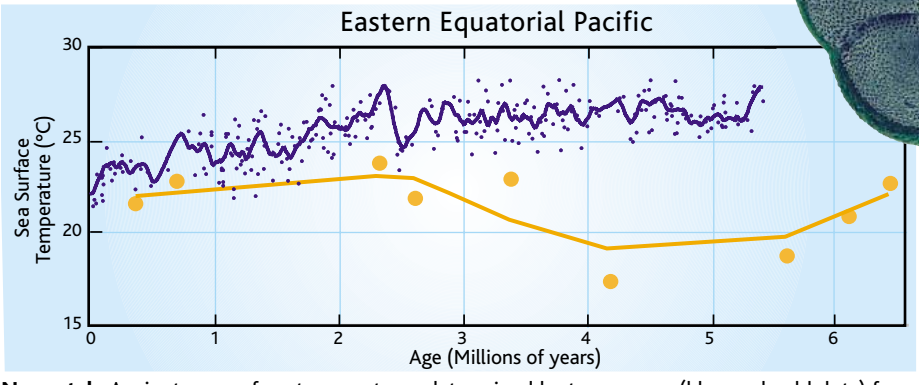
El Niño or La Niña? The Past Hints at the Future

Two teams of researchers, studying the same evidence with the same techniques, have painted diametrically opposite pictures of a key period in the history of Earth's climate, which climatologists are probing for hints of what's to come. "It's a tough issue to sort out," says climate modeler Raymond Pierrehumbert of the University of Chicago in Illinois. "What's at stake is the regional distribution of climate," both past and future. But he's going to have to wait for more data from the past.

The two groups, one British and one American, are studying what temperatures in the equatorial Pacific Ocean were like during the early Pliocene epoch, about 4.5 million to 3.0 million years ago. The world was about 3°C warmer then than it is today—much as it may be a century or two from now. Today, the tropical Pacific is the "engine" that drives much of the global climate system. Computer climate models disagree about how future global warming will affect it: whether the region will get stuck in the warmth of a permanent El Niño, slip into the relative cool of an endless La Niña, or keep swinging from one to the other as it does today. By showing how the tropical Pacific worked the last time the world got hot, climatologists hope the Pliocene will help them forecast what to expect next time.

To find out ancient ocean temperatures, each group studied a pair of deep-sea sediment cores from either end of the pivotal equatorial Pacific, one taken from near the Galápagos Islands and one from 13,000 kilometers to the west. From the mud, they extracted the fossils of microscopic creatures called foraminifera, or forams, that lived in Pliocene surface waters and sank to the bottom after they died. By studying the ratio of the elements magnesium and calcium preserved in forams' carbonate shells, scientists can estimate the temperature of the water the creatures once floated in.





No match. Ancient sea surface temperatures determined by two groups (blue and gold dots) from forams (*inset*) starkly disagree during the early Pliocene (3.0 million to 4.5 million years ago).

SOURCES: M. W. WARA *ET AL.*, *SCIENCE*; R. E. M. RICKABY AND P. HALLORAN, *SCIENCE*; (INSET) DEE BREGER/DREXEL UNIVERSITY

The British group weighed in first (*Science*, 25 March, p. 1948). Rosalind Rickaby and Paul Halloran of the University of Oxford, U.K., published six eastern Pacific temperatures spanning the past 5 million years, including one from the Pliocene warm period. It showed that the eastern Pacific was dramatically cooler than the west—the hallmark of a dominant La Niña.

Now, on page 758, the American group—Michael Wara, Christina Ravelo, and Margaret Delaney of the University of California, Santa Cruz—reaches a different conclusion. They produced more than 200 temperatures over 5 million years, including more than 50 from the time of Pliocene warmth. Wara and colleagues conclude that at that time the eastern Pacific was only slightly cooler than the west. The implication: El Niño, not La Niña, ruled the early Pliocene.

It's a big difference. A dominant La Niña would have made the world slightly cooler on average than the alternative. More important, La Niña's regional climate effects—such as a wetter western Pacific and a cooler northwestern North America—would have been felt around the globe. If El Niño prevailed, on the other hand, that would have meant a warmer climate overall and much warmer and drier conditions in southern Africa, for example.

So who is right? Outside experts say the Californians' hundreds of temperature readings give El Niño a tentative edge. "You need really dense data sets to do this work well, in my opinion," says paleoceanographer David Lea of the University of California, Santa Barbara. "This is difficult work, and it's easy to be misled." Paleoceanographer Gary Dwyer of Duke University in Durham, North Carolina, agrees, noting that sampling as sparse as the Oxford group's could make it easy to mistake a few rare cold-water interludes for a long-term La Niña regime. But Rickaby stands by her team's results and hints that superior British sample cleaning more than closes the numerical gap in data points.

Researchers say only more research can settle what really happened during the Pliocene. "There may be missteps before it's done," says Pierrehumbert, but "I can't overemphasize the importance of such data" to testing climate models **–RICHARD A. KERR**

The ambitious Northwest Forest Plan tried to balance desires for timber and biodiversity, but preservation trumped logging—and research. Can the plan be made as adaptable and science-friendly as intended?

Learning to Adapt

For decades, a steady stream of logging trucks rolled out of forests in the Pacific Northwest, piled high with ancient Douglas firs, valued for their huge trunks. Old-growth forests on private lands were the first casualties, and as they disappeared, the loggers turned to national forests. Despite outcries from environmentalists, the pace of clear-cutting intensified in the 1980s—reaching a peak of more than 5 billion board feet a year, enough to build 350,000 three-bedroom houses, much of it from old growth. Then in the early 1990s, environmentalists finally found a weapon powerful enough to fight destruction of these venerable forests: the northern spotted owl, which needs large tracts of old trees to survive.

Not long after the owl was added to the endangered species list in 1990, environmental groups sued on its behalf, and a federal judge ordered a moratorium on logging in owl habitat. The rumble of trucks from the national forests silenced, but the volume of the debate only got louder. As it played on national media, the bitter battle pitted birds against jobs. Activists spiked trees to damage mills, while loggers held protests and cut down old-growth trees at night. The tension ratcheted up.

Out of this political crisis came the largest, most ambitious forest conservation plan ever. Called the Northwest Forest Plan (NWFP), it covers 9.8 million hectares of federal land in California, Oregon, and Washington. Striving for compromise, the plan tried to balance the needs of loggers and endangered species. To meet that tall order, the architects set up special research areas to devise new ways of cutting timber that would be benign or even beneficial to wildlife. Economic and ecological progress would be monitored, and the plan would be altered decade by decade as needed—a process called adaptive management.

Now, more than 10 years and $50 million in monitoring costs later, researchers and forest managers have taken the first major stab at assessing how well the plan is working. This fall, they will publish a series of extensive reports, with a synthesis slated for release this month. The bottom line, they say, is that the plan is basically on track: Old-growth forest has been preserved, and watersheds are improving. But several key goals have not been met. Some forests face the risk of catastrophic fires; the spotted owl population is still declining; and timber sales never came near projections, meaning lost jobs and dollars for both the timber industry and the U.S. Forest Service (USFS).

Flash point. Cutting of old-growth trees, like this Douglas fir, created bitter conflict and led to the Northwest Forest Plan.

Another shortcoming is the relative dearth of new approaches for improving the plan. Despite good intentions, the goal of devising and studying alternative management strategies essentially fizzled. Officials say that fixing this is a top priority, as is reducing fire risk.

But keeping the plan on track—let alone boosting its activities—faces serious challenges, as funding for the USFS in the Pacific Northwest has fallen dramatically. Forest service officials say that changes in regulations governing the plan, implemented by the Bush Administration, will give them needed flexibility, but environmentalists worry that the changes provide license for irresponsible logging that could threaten remaining old-growth forests.

Legal logjam

Several broad environmental laws passed in the 1970s made the conflict between logging and old-growth conservation all but inevitable. The Endangered Species Act (ESA) of 1973 requires the conservation of habitat that listed species depend on, and sections of the National Forest Management Act mandate that populations of species be kept viable. Forest service officials knew in the 1980s that the spotted owl was likely to be listed but, under pressure from politicians in the northwest, continued to allow cutting of old-growth forests—until the Seattle Audubon Society and other groups sued.

In March 1989, a federal circuit judge blocked sales of timber within the range of the owl, an area encompassing the remaining old growth. Congress intervened, allowing a few timber sales to go through, enraging environmentalists. The issue rose to prominence in the 1992 presidential campaign.

A few months after the election, President Clinton asked a large group of scientists from USFS, the Bureau of Land Management (BLM), and universities to provide a range of options that could end the judicial moratorium. The Forest Ecosystem Management Assessment Team (FEMAT) was charged with finding ways to protect the long-term health of the forest across the range of the spotted owl while providing "a predictable and sustainable level of timber sales and nontimber resources that will not degrade the environment."

A core team of several dozen researchers, led by wildlife biologist Jack Ward Thomas of USFS, holed up for 3 months in a Portland office building, working around the clock and calling on more than 100 outside scientists when needed. "The mood was one of great

CREDITS (TOP TO BOTTOM): PHILIP MCDONALD/USDA; GETTY IMAGES

intensity and focus," says FEMAT participant Norman Johnson of Oregon State University in Corvallis. From this came a 1366-page document that laid out 10 distinct management options. All of them took a broad view, focusing on managing the entire ecosystem rather than just the spotted owl. But to survive court challenges, any plan had to comply with laws aimed at species protection.

Clinton picked Option 9, which set up a patchwork of old-growth areas—45 so-called Late Successional Reserves, totaling 2.8 million hectares or almost 30% of federal land in the plan area. The primary objective in these reserves was to ensure the survival of old-growth forest habitat that the owl requires. Some 1.9 million hectares outside the reserves, called the matrix, would be available for logging, except near owl nests.

To figure out what type of management would be most compatible with conservation and timber goals, the plan set aside 10 areas (see map, p. 690), totaling 603,000 hectares, for experimentation with restoration and harvesting approaches. It also called for different management strategies in various reserves, depending on local conditions. For instance, the pine forests east of the Cascade Range are drier and more prone to fire than those to the west, and decades of fire suppression had led to a buildup of brush and deadwood. They would need aggressive management, including thinning and prescribed burns, to prevent catastrophic fires. To the west of the mountains, by contrast, the idea was to accelerate the development of old-growth habitat by thinning second-growth plantations.

Because officials expected salmon to be listed under ESA, the plan also includes a substantial Aquatic Conservation Strategy. To prevent erosion, which adds sediment and can destroy fish habitat, the plan creates a system of riparian reserves: 100-meter-wide no-logging strips on either side of streams, totaling 903,000 hectares. As more was learned about watershed ecology, the buffers were to be adjusted to the minimum size necessary to conserve fish, thus allowing more logging.

Before it was implemented, Option 9 went to the departments of Interior and Agriculture, where it was modified—presumably to make it legally more airtight—without scientific advice from FEMAT. The biggest change was to expand the scope of protection beyond species listed under the ESA to include several hundred largely unstudied species whose status was unknown. "The precautionary principle went berserk at that point," Thomas says.

Under this additional "survey and manage" program, before any ground-disturbing activity could take place, the agency had to check for the presence of any of these organisms, including lichens and invertebrates, and devise a plan to minimize impact on them. Although this provision has helped the overall plan hold up to court challenges, it had unintended and wide-ranging consequences. In particular, because it made the plan substantially trickier to implement, much logging and many adaptive-management experiments never got off the ground. "It almost made it impossible to pursue the actions in Option 9," says Thomas, who was chief of USFS from 1993 to 1996.

Northwest Forest Plan: A Decade Later

OLD GROWTH
Despite forest fires, the plan area ended up with slightly more old forests than expected. Only 0.2% of old growth was logged, but critics say even that was too much.

SPOTTED OWL
Likely reasons for population declines include competition from barred owls and habitat loss on private land.

MARBLED MURRELET
Populations of this endangered seabird were expected to decrease by 35% but apparently remained stable for unknown reasons.

RIPARIAN HABITAT
Some 4800 kilometers of roads were decommissioned and deadwood added to streams to improve fish habitat.

TIMBER AND ECONOMICS
Lawsuits and complex regulations meant far less timber, little improvement in fire risk, and slower maturation of managed forests. Some towns suffered seriously, although the region prospered overall.

ADAPTIVE MANAGEMENT AREAS
Most research sites never saw much action, due to lawsuits, bureaucracy, and limited funding.

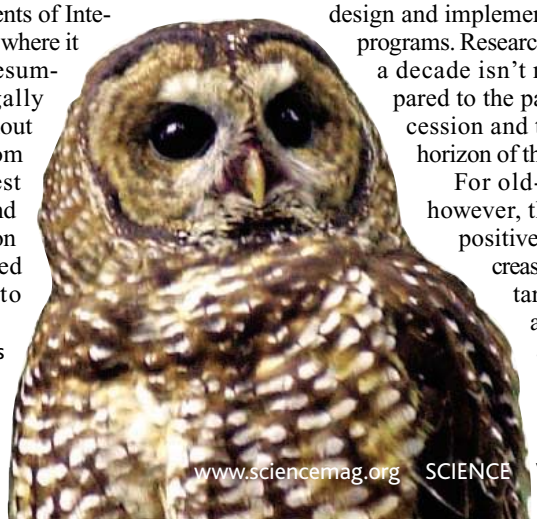
Charting progress

This spring, USFS and BLM began previewing the first monitoring results. In some cases, the data are too sparse to yield a useful assessment, because it took several years to design and implement the monitoring programs. Researchers also note that a decade isn't much time compared to the pace of forest succession and the century-long horizon of the plan.

For old-growth forests, however, the trend appears positive. Older forest increased by 245,000 hectares between 1994 and 2003, about the amount originally expected. "Perhaps we can conclude for the short term that the policies are working," says USFS's Melinda Moeur, who led the old-growth monitoring team. But environmentalists counter that the net increase—tabulated when an average tree diameter crosses a certain threshold—means only marginal improvement in habitat, while the 6800 hectares of older forest that were clear-cut represent real setbacks. "The losses are catastrophic, while the gains are incremental," says Doug Heiken of the Oregon Natural Resources Council in Eugene.

The plan fell far short of its goal in terms of timber production. About 0.8 billion board feet per year were expected to be put up for sale each year; in most years less than half of that was. A major factor was the stringent requirements of the "survey and manage" program. Environmental groups also slowed things down with lawsuits to prevent any harvesting they thought detrimental.

This decline in timber harvesting had both economic and ecological effects. Although it cost roughly 23,000 timber-related jobs, that was less than some had feared. Jobs with USFS also disappeared and were not replaced. Yet over the decade, some 800,000 other jobs were created in the region. As former timber workers and USFS employees moved out, they were replaced by retirees and telecommuters. Overall, the Pacific Northwest did not suffer economically because of the plan, says forest economist Richard Haynes of USFS, but some rural communities were hit quite hard. The shortfall of cutting also has ecological implications. The paucity of clear-cutting in former plantations, which would mimic the effects of a severe windstorm or major fire, means that the northwest could end up many decades from now with a lack of early succes-



Decline. Spotted owls face competition from an invasive species.

CREDIT (BOTTOM): ASSOCIATED PRESS

sional forests, which are prized for their biological diversity. And because there was little thinning, which both provides timber and helps accelerate forest succession to old growth, the fire hazard continued to increase in eastern old-growth forests.

Another disappointment is that despite the progress in habitat preservation, the population of spotted owls is estimated to be declining at 3.4% per year. The culprit is a surprise: invasive species. Barred owls, which are native to the central and eastern United States, have moved west over the past few decades. The newcomers seem to dissuade spotted owls from hooting, and spotted owls are apparently more likely to leave their territory if barred owls appear. Moreover, their diets overlap 75%, so they may be competing for food as well. "Barred owls may ultimately be as big or bigger a threat than habitat loss," says Eric Forsman, a wildlife biologist with USFS in Corvallis.

Thin is in. Selective logging can speed forest maturation, reduce fire risk, and produce timber.

Trying to adapt

A cornerstone of the original plan was adaptive management—essentially, learning by doing and monitoring—which had never been tried on this scale before. The plan called for setting aside 10 adaptive-management areas (AMAs), where scientists would test ideas about how to create or restore forest or riparian habitat and protect threatened species while integrating timber harvest. Most never got off the ground, which leaves the Forest Service with few new ideas to guide efforts to improve the plan. "It's been an extremely frustrating decade," says forest ecologist Bernard Bormann of USFS. "The progress has been very slow."

Several factors scuttled the projects. Tension and lack of trust between forest managers and environmental groups figured large. When environmental groups felt that foresters were using AMAs primarily to extract timber rather than to improve the ecosystems, they sued. However, Dave Werntz of the Northwest Ecosystem Alliance in Bellingham, Washington, says that trust has been building, thanks to better communication and good-faith efforts: "We're doing a better job today at implementing the Northwest Forest Plan than any time in the past."

Other problems remain: When national forest budgets got tight, these experiments were axed or fell lower on priority lists. In addition, rather than being encouraged to try novel approaches, local managers had to offer evidence to the U.S. Fish and Wildlife Service (FWS) that experiments wouldn't harm listed species. In many cases, managers simply gave up trying to make projects work or walked on eggshells to avoid legal trouble. "Caution seems to have trumped creativity," says Elaine Brong, BLM's director for Oregon and Washington.

There were a few exceptions. The Blue River Adaptive Management Area, for instance, was set up to recreate the effects of historical patterns of forest fires across

Mixed success. Old growth was preserved on federal land, but not many experiments took place.

23,000 hectares in the Cascades near Eugene, Oregon. Cutting, combined with prescribed burns, has yielded timber at a low but constant rate. The project began only 5 years ago, so no results have emerged yet. But modeling indicates that the experiment will create more old forest than the standard design of the NWFP will and much more intermediate-age forests. "We'll end up with what we believe is a more natural system," says geomorphologist Fred Swanson of USFS. And thinning experiments in the Siuslaw National Forest near Waldport, Oregon, are probing the best way to accelerate the maturation of younger forests, says Bormann, the lead scientist. Thanks to the thinning, the Siuslaw now produces more timber than any other national forest in the NWFP.

Overall, scientists say the plan is succeeding at its goal of conserving old-growth ecosystems. "So far so good," sums up Thomas Spies, a forest ecologist with USFS. Conservation wasn't the exclusive goal at the outset, of course, but the agency seems resigned that it won't meet its timber harvests. "If we can keep them flat, then we'll be doing pretty good," says USFS spokesperson Rex Holloway.

That state of affairs—if it holds—distresses the timber lobby but pleases environmentalists. The Bush Administration has, however, implemented several changes that could swing the balance, such as eliminating the "survey and manage" requirements last year to boost timber production. Other major changes, which affect all national forests, include removing the concept of retaining viable populations from the National Forest Management Act and lessening mandatory monitoring and requirements for environmental-impact statements. The changes "give total discretion to the local forest manager on how to manage the forest," says Michael Leahy of Defenders of Wildlife in Washington, D.C., which has filed suit.

How these changes specifically affect the operation of the plan will be determined by the Regional Interagency Executive Committee (REIC), made up of officials from USFS, BLM, and other agencies. This group will also decide how to modify the plan based on what's been learned over the past decade. A key priority is "getting the AMAs to work," says Linda Goodman, regional forester of USFS's Pacific Northwest Region and a REIC member. One strategy is increased involvement of FWS and the National Oceanic and Atmospheric Administration's National Marine Fisheries Service, which are responsible for endangered species, in research design so that scientists and managers have more latitude to take risks.

Yet as they hope to ramp up research and management activities for the next decade, Forest Service managers face a declining budget and downsizing. The agency's budget dropped 35% in the NWFP area during the first decade, which forced it to cut 36% of positions and close about 23% of its field offices in the plan area. "I'm very concerned," says Jerry Franklin of the University of Washington, Seattle. "What's happening is a real threat to carrying forward the plan successfully." To a large extent, the question of funding will determine how much monitoring and experimentation will continue—and what researchers will have learned about managing the forests 10 years from now.

—ERIK STOKSTAD

CREDITS: USFS PACIFIC NORTHWEST RESEARCH STATION

New National Academy Head Is No Stranger to Spotlight

Ralph Cicerone came to Washington, D.C., this month to lead the National Academy of Sciences—and walked smack into a hot climate debate

Last week, Ralph J. Cicerone showed the U.S. Senate what he might be like as the new president of the National Academy of Sciences (NAS): a politically savvy administrator who intends to make the voices of scientists heard in Washington, D.C., and beyond.

On consecutive days, the 62-year-old atmospheric scientist testified before separate panels examining the science of climate change. To the first panel, he explained firmly why the National Academies had waded into a fight brewing between an influential House committee chair and scientists whose research has linked rising temperatures with human causes by volunteering to look into the questions that Representative Joe Barton (R–TX) had raised about Michael Mann's work (*Science*, 22 July, p. 545). In the second, he addressed a legislator's concerns about the economic costs of capping greenhouse gas emissions by ticking off seven ways in which efficient energy use would help average Americans.

Colleagues say his performance, scarcely 2 weeks into his 6-year term as NAS president, was typical of someone who knows how to talk to politicians, peers, and the public. "He's very good at putting all the pieces together from different disciplines to provide a simple answer for societal questions," says atmospheric chemist Guy P. Brasseur of the Max Planck Institute for Meteorology in Hamburg, Germany.

Policy-oriented answers to complex problems are the academies' stock in trade. More than 200 times a year, it delivers measured judgments on issues from teaching evolution to energy policy. In 2001, while still chancellor of the University of California (UC), Irvine, Cicerone himself chaired a White House–requested academies' review of climate science that said human activities could result in higher temperatures, drought, and increased rainfall while noting uncertainties. "We were all on the hot seat," says botanist Peter Raven, who led the academy committee that nominated Cicerone to succeed Bruce Alberts. "But he really came through, with rigor and accuracy."

Although Cicerone called his back-to-back Senate appearances "probably more than I'd like to do," a busy, high-profile schedule is hardly a novelty for him. He maintained a productive research lab at Irvine during his 7-year stint at the helm, avoiding serious cuts to programs and personnel despite a tough budget environment. Raven says that Cicerone's public relations and fundraising skills helped him nab the NAS job.

Cicerone began his career as an electrical engineer studying atmospheric plasmas. At the University of Michigan, Ann Arbor, in 1973, he and Richard Stolarski showed that free chlorine atoms could decompose ozone catalytically, earning the pair a citation when UC Irvine colleague Sherwood Rowland won the Nobel Prize in 1995. His interests steadily broadened, from methane's role in greenhouse warming to climate change, and he reported his findings in regular testimony on Capitol Hill.

Hot seat. New NAS President Ralph Cicerone prepares to testify at a Senate hearing on climate change.

Cicerone spoke last week with *Science* about his new job. Here are excerpts from that conversation. –ELI KINTISCH

On his goals for NAS:
"In my lifetime, I think I've seen a pretty pronounced slippage of the public's enthusiasm for and understanding for science. And I'm going to try to get a number of academy members together and some of our staff to look at our past efforts on communicating and see what we can do better. …

"I'm [also] really worried about the U.S. science and technology base. … We have a couple of groups working right now to assemble some measures of how we track our progress and our relative standing around the world. … We'll be working this one with the National Academy of Engineering and with scientific and engineering society leaders, too."

On the timeliness of reports:
"That's always been a criticism, but I think things have sped up a little bit. … There have been some fast ones lately, like what to do with the Hubble [Space Telescope]. … You couldn't take on the number of studies we're doing now if all of them were, let's say, 2-month turnaround. And I think by nature, many of the questions we're asked to look at are longer term, anyway."

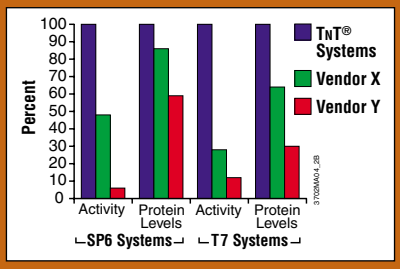
On the number of women members:
"Last year's [entering class] was the all-time record, with 19 out of 72. … We're doing better, but there are still a lot of ways in which women are not being involved enough, like in our choice of award winners and officers of the academy. We've got a long way to go."

On his career progression:
"I think there's a real difference between leadership and management and administration. … [In 1994] we had a fantastic dean of physical sciences who had to step aside for personal reasons, and they asked me to take over the job. I was out of town when the faculty met. … [But] I've always enjoyed trying to do several things at once. Then when the opportunity came to be chancellor of the campus, … someone said to me, 'You've complained a lot at the way other people do these jobs. Maybe it's time for you to try it.'"

On a funding gap between the life and physical sciences:
"In the physical sciences, I think there are many discoveries out there waiting to happen, largely because of our new capabilities in measurement. … I think it was necessary to increase the portfolio for biological and health sciences, and I'm really glad we've done it. But the physical sciences have fallen too far behind."

CREDIT: CHRIS MADDALONI

TnT® Cell-Free Protein Expression. Build on it!



Generate more functional protein with Promega.

Easy, one-step protocols provide quick and accurate results. Use Promega TnT Coupled Transcription/Translation Systems. Generate more functional protein than you'll find in other available eukaryotic-based systems.

Ideal for:

- Protein:protein interactions
- Protein:nucleic acid interactions
- In vitro expression cloning

To learn more visit **www.promega.com/cellfree**

PROMEGA CORPORATION • www.promega.com

Promega

Tackling the Cancer Genome

Genome sequencers and cancer experts hope a pilot NIH project to find genetic glitches in tumors will build support for a complete catalog of human cancer genes

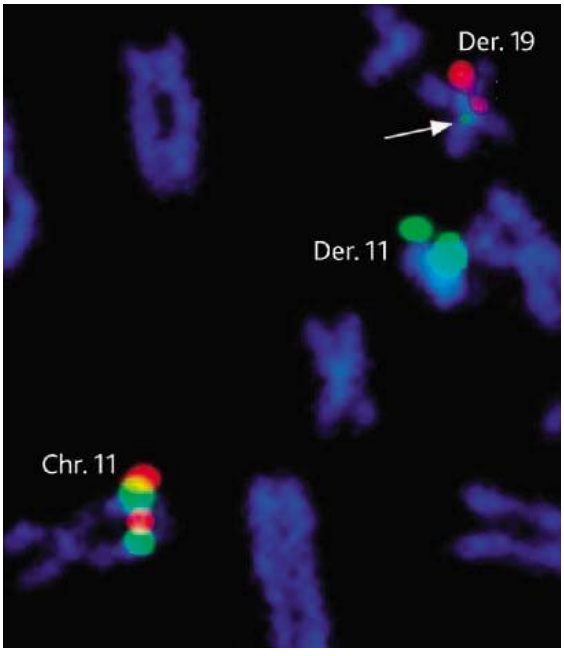
The scientists who brought you the human genome project are teaming up with cancer researchers for another big-biology moon shot. They want to compile a catalog of all common mutations found in human cancers, with the goal of jump-starting molecular approaches to treating cancer. Last week, at a Washington, D.C., workshop to explore the idea, scientists sketched out a game plan for a 3-year pilot project. And two institutes of the National Institutes of Health (NIH) in Bethesda, Maryland, announced a $100 million down payment on a project expected to cost $1.5 billion over a decade.

The human cancer genome project was hatched by a group of advisers to the National Cancer Institute (NCI) led by Eric Lander of the Broad Institute in Cambridge, Massachusetts, who unveiled the initial plan in February (*Science*, 25 February, p. 1182). It would identify the genetic glitches that lead to uncontrolled cell growth in most cancers. Lander's group proposes systematically searching for the common mutations in 12,500 tumor samples from 50 major cancer types. "If everybody were to pull together, we could at least know the enemy in a decade," Lander says.

Planners say the project dovetails with the push by NCI Director Andrew von Eschenbach to translate genomic discoveries into the clinic. Von Eschenbach says the idea "will embed in the entire strategy of the NCI," which has agreed to share the cost of the pilot project with the National Human Genome Research Institute. The full project, however, would require additional funding from Congress.

Cancer genome project backers hope to repeat the success of the human genome effort. But determining the sequence of the 3 billion bases in human DNA, although controversial at first, was a well-defined task compared to what cancer researchers are proposing. The new project would collect samples from thousands of patients, analyze those samples for mutations found in at least 5% of cases, and measure gene-activity patterns in the tumors.

Because a full sequencing of each sample to find mutations would cost too much, Lander's group has proposed at first sequencing only the coding regions of 2000 or so genes implicated in cancer. Even then, much of the data may be meaningless, notes Michael Stratton of the Sanger Institute in Hinxton, U.K., which has a smaller cancer genome project under way. Stratton presented data at the workshop on protein kinases, enzymes involved in cell signaling, for several cancers. Only a small fraction of mutations in kinase genes cause abnormal cell growth, he reported, and although some tumor samples carried several mutations, others had none.



Know the enemy. A new cancer project will look for genetic changes, such as this chromosomal translocation in a salivary gland tumor.

Given the uncertain results from sequencing, cancer researcher Ronald DePinho of Harvard University pushed for analyzing gross genetic changes that are relatively easy to detect and known to lead to cancer, such as extra copies of genes and chromosomal translocations. "That might be the quickest way to get the most bang for the buck," DePinho says. Other workshop participants suggested that mutations in regulatory regions—which determine how much of a protein is produced—could prove even more important than coding regions. And some urged using emerging technologies to take a closer look at epigenetics, such as changes in DNA methylation patterns that affect whether genes are turned on or off.

Scientists also debated exactly where to begin the pilot. Some argued that an in-depth analysis of one type of cancer would be more likely to hit a home run—for example, find a mutation that flags which patients would benefit most from a particular treatment. But others argued that studying several cancers would boost the odds of a treatment breakthrough and keep more patient advocacy groups on board. "We need deliverables," said Bruce Stillman, president of Cold Spring Harbor Laboratory in New York.

All this testing will require large amounts of tumor tissue with reliable clinical information attached and proper consent from patients. Because genes expressed in tumors change over time, scientists may need to test tumors at different stages. Attendees pondered whether to collect new samples over several years or to rely on existing tissue banks, assuming the researchers who collected them are willing to share. They also worry about community resistance to making the data freely available quickly. That provision may give some cancer researchers "the heebie-jeebies," said one speaker.

Despite the challenges, workshop participants agreed to start by focusing on a few tumor types drawn from existing samples. Tissue banks will be invited to participate later this year, and the best proposals will determine which cancers to study. Meanwhile, the pilot will also begin developing methods for collecting new samples for later—and presumably cheaper—analysis. Other requests for applications will seek proposals for technologies, both high-throughput sequencing at genome centers and small-lab techniques such as microarrays for expression analysis.

To succeed, proponents will need lots of friends from a research and advocacy community that may have doubts not only about the project's eventual price tag but also about the value of fishing for data rather than investigating a hypothesis. "We have a lot of questions," says Fran Visco, president of the National Breast Cancer Coalition in Washington, D.C., which is still studying the idea. "How are we going to prioritize so it's not creating data to keep scientists busy and not really helping patients?" That's one of many concerns scientists must address to make the cancer genome project a success.

–JOCELYN KAISER

CREDIT: *NATURE GENETICS* **30**, 208-213 (2003)

Strong Personalities Can Pose Problems in the Mating Game

A closer look at confrontational behavior in various animals shows that aggression may help individuals survive, but it can impair reproductive success

For male fishing spiders, courtship is dangerous business. Females of the species are notoriously aggressive, and the male—which signals his arrival by gently tapping the surface of the water—often ends up as a meal rather than a mate. Yet each time the female eats her would-be partner, she lessens her chance of reproducing, leaving evolutionary biologists wondering just why this behavior persists. Aggressive female spiders just can't stop themselves, says J. Chadwick Johnson, a behavioral ecologist at the University of Toronto, Scarborough.

Johnson is among a small group of researchers investigating the "personalities" of animals from spiders and fish to insects and birds. Although many biologists once strongly protested attributing human qualities such as personalities to animals, more and more investigators are adopting such descriptive language. Individual animals, even simple invertebrates, do have consistent behavioral quirks that endow them with discernible dispositions, says Andrew Sih, a behavioral ecologist at the University of California, Davis.

Although he and his colleagues think of these dispositions as personalities, they have tried to steer clear of being criticized as anthropomorphic by instead coining the term "behavior syndromes." In addition to identifying such syndromes in animals, Sih, Johnson, and several other investigators are finding that animal personality traits, such as being bold toward potential predators or aggressive toward cohorts, can have drawbacks, despite the traits' apparent value, say in hunting or defending territories. For example, Renee Duckworth of Duke University in Durham, North Carolina, has shown how one bluebird species' aggressiveness allows it to steal habitat from another—yet that same trait impairs the bird's reproductive fitness in certain conditions. Looking at animal personalities, and the good and bad they bring, represents "an important paradigm shift in our approach to the evolution of behaviors," says Duckworth.

Eight-legged dominatrix. A female fishing spider devours her suitor.

Dangerous liaisons

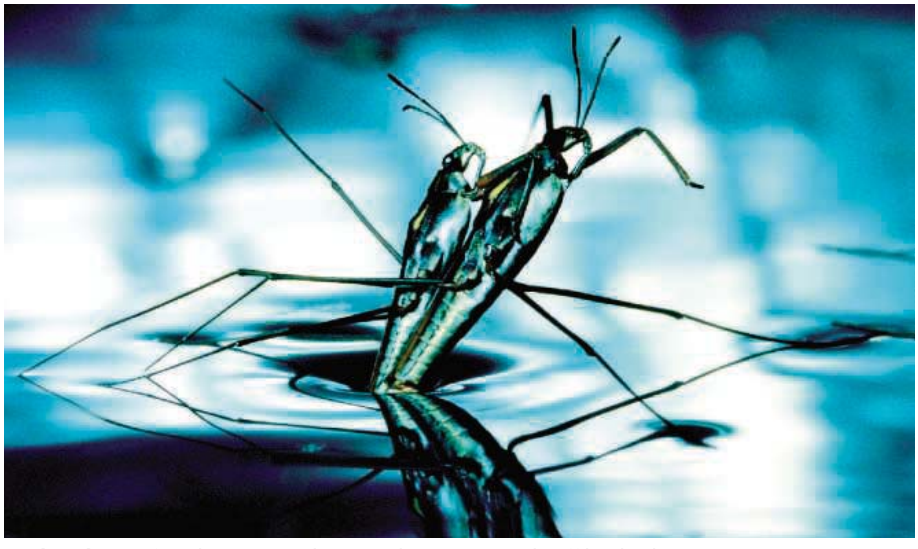
Many researchers credit Sih for bringing to prominence the idea that animal personalities carry survival risks. The notion plays off a proposal made 25 years ago by the late paleontologist Steven J. Gould and geneticist Richard Lewontin, both from Harvard. At that time, the two stirred up the evolutionary biology community by arguing that maladaptive traits could persist if they were linked with beneficial ones in an often-precarious balancing act. For example, guppies living around predators reproduce as early as possible so as to pass on their genes before being eaten. But the eggs slow gravid females down, making them easier prey earlier in life, a finding that lent credibility to Gould and Lewontin's idea.

Now, by showing that a personality trait that is counterproductive in one context perseveres because of its utility in another, Sih is moving Gould and Lewontin's ideas "into a new arena," says evolutionary ecologist Andrew Hendry of McGill University in Montreal, Canada. Sih argues that because some animals are very limited in their ability to moderate their personalities according to particular situations, they are stuck with the consequences throughout their daily lives.

Take the North American fishing spider, the subject of Johnson's studies. In 1997, Göran Arnqvist of Uppsala University in Sweden and a colleague suggested that aggressive females who eat males who come courting were simply following their strong instincts to catch prey. The drive to hunt would serve juvenile females quite well, enhancing their growth, particularly when competition for food was intense. But those instincts, if unfettered, may backfire when the females become adults and need mates.

Johnson has recently followed up on this proposal, verifying key elements. He found that even as young spiders, certain females were aggressive hunters, spending more time than their cohorts searching for the next meal and, as a result, bulking up more. This aggressiveness was also reflected as boldness in encounters with predators, Johnson discovered when he mimicked a bird's approach by tapping the water near these spiders. Although all fishing spiders dove into the water when they detected such tapping, the female superpredators surfaced more quickly.

These daredevils also were more likely than less aggressive females to try to snack on males, Johnson reported last month at Evolution 2005 in Fairbanks, Alaska. "Boldness to a simulated predator is proportional to the tendency to attack males," he said. Overall, he concluded, the bold, aggressive female spiders ate more food, but they compromised their survival and productivity by treating males as food and taking predation risk lightly.



No love lost. A female water strider struggles to get a male off her back.

CREDITS (TOP TO BOTTOM): NANCY A. KREITER; JENS RYDELL AND INGELA DANIELSON

Daniel Promislow of the University of Georgia, Athens, is surprised that aggression can pervade all aspects of a female spider's life. If the fishing spiders could modulate their personality, he explains, then the females should be as aggressive as possible in hunting, less aggressive in the face of danger, and mild-mannered when approached by males—but that's not what the experiments indicate. "We often think of behaviors as relatively plastic traits compared to morphology, physiology, or life history," he says, but Johnson's results challenge that premise.

Counterproductive aggression is not limited to female arachnids. Sih has found that militant males are the troublemakers among insects known as water striders. Sih graded aggressive tendencies in males by observing, for example, how much they fight, how long they were active, and how often they chased after potential female mates. He then put together 12 groups of water striders, each consisting of males with similar personalities from least aggressive to most aggressive, in separate artificial ponds. The researchers then put females into the ponds and monitored each group's mating successes and failures, keeping track of each individual's partners within their group. The investigators also tracked each water strider's feeding and tallied how often an individual retreated to riffles, supposedly a more dangerous habitat but also a refuge from aggressive peers.

Females tended to avoid the most aggressive males, the researchers found. Indeed, females often refused to put up with any "Rambo" male in their midst and moved as far away from him as they could, diminishing both his and his peers' mating opportunities. Aggressive individuals couldn't turn down their swagger. They ultimately "hurt not only themselves but, by being too aggressive, the entire group," Sih reported at the evolution meeting.

Group dynamics

Working with small fishes called threespined sticklebacks, Alison Bell of the University of Glasgow, Scotland, has found that living conditions may narrow the range of personalities within a group of animals. Whereas researchers such as Sih and Johnson typically focus on the behavior of individuals in a population, she is assessing variation in "in your face" behavior—the combination of boldness and aggression—between and within whole populations of the fish. Because stickleback populations have diverged genetically, so might their behavior in different places, she hypothesized.

To examine this possibility, Bell collected groups of 20 juveniles from 13 different populations of freshwater and marine sticklebacks in various lochs and harbors around Scotland. Some of these populations regularly faced predators—pike, trout, and the like—and others lived in relatively predator-free environments. To measure boldness of the fish from each population, she set up a tank with a pike behind a glass divider, then counted how often individual fish approached the pike to inspect it. For a gauge of aggressiveness, she counted the number times a fish isolated in one tank tried to nip at other sticklebacks in an adjoining tank separated by glass.

The fish within each of the 13 populations seemed to share similar mindsets. Bell found that when one individual from a population fearlessly approached the pike, so did most of the others from those groups. In general, most of the fish within a particular group acted the same way, she reported. And fish from the boldest populations, as measured by the pike test, were also the most confrontational toward other sticklebacks. In the wild, says Bell, this bullying could translate into bigger territories, better food, and even increased mating for the biggest bully. But the fearlessness toward predators may also cost fish in these aggressive groups their lives, suggesting that whole groups of animals, not just individual ones, can have personality traits that threaten reproductive success at times.

Bell also observed that the bold, aggressive stickleback populations had higher breathing rates, more spines, and heavier body armor than more wimpy populations. Those correlations suggest that "behavioral syndromes might be part of a larger package of evolutionary [traits]," says Sih.

Duckworth's studies indicate that sometimes the bold personality of one species can help it beat out similar, but shyer, species, at least in a particular environment. Observations over the past 40 years show that western bluebirds have greatly expanded their range in Montana, displacing mountain bluebirds. By tallying the number of each bluebird in places where both species are present, Duckworth documented that western bluebirds in just a few years supplanted mountain bluebirds at valley study sites. Much of the western bluebird's success sprang from its fierceness, suggests Duckworth.

She placed tree swallows, a bluebird competitor, in nest boxes, and then watched as either of the bluebird species approached the box. She found that western bluebirds were more aggressive, an indication that they are better able to acquire and defend their territories against the swallows. The male western bluebirds also were fiercer than mountain bluebirds when competing for mates, another sign of pushy temperaments.

In this case, aggressiveness seems to go hand in hand with reproductive success. But a closer looks suggests that, as with fishing spiders and water striders, the western bluebird's obnoxiousness can come with a cost. Duckworth points out that western bluebirds spend so much time defending their nests and courting that they neglect their offspring. This poor parental behavior is especially problematic in tough environments, such as mountains. In contrast, mountain bluebirds are loyal parents and have an edge where weather can be rough, says Duckworth. As a result, they have maintained their foothold in Montana's mountains. "Behavioral syndromes can have profound ecological and evolutionary consequences by mediating species coexistence," Duckworth says. Thus, in animals, as in people, personality can make or break one's success in life.

–ELIZABETH PENNISI

Buzz off. A test of aggression shows that western bluebirds are quite fierce against swallows.

CREDIT: ALEX BADYAEV

Random Samples

Edited by Constance Holden

Updated Cranium

Below is the latest model of the skull of Kennewick Man. Because scientists aren't allowed to make casts from the original bones, it was produced this month from hundreds of high-powered computed tomography scans. On 15 July, an 11-member team headed by anthropologist Douglas Owsley of the Smithsonian Institution wrapped up 10 days of preliminary study of the 9400-year-old remains, held in the Burke Museum in Seattle, Washington.

Owsley says the studies promise to yield even more information than he expected and in fact "are going to take us to a level that has never been done with another skeleton." In this first round of study, which Owsley calls "a full-blown taphonomic analysis," the scientists have been able to ascertain when and how bone fractures occurred as the skeleton lay in the ground. Thus, he says, "I'll be able to tell you how the hands and feet were positioned," and therefore "we will know whether this was a burial."

The next visit to the bones, which will probably occur early next year, will entail a new cast of scientists who will do pathology and investigate Kennewick Man's lifestyle. Owsley also says the age at death will probably be revised and the time of Kennewick's demise narrowed from the current 2700-year window.

NIH's Public Access Trickle

Last year, a huge scuffle broke out over a National Institutes of Health (NIH) plan to ask grantees to submit their accepted papers to a free archive. Open-access advocates hailed the move, whereas journals said they would be bankrupted (*Science*, 3 September 2004, p. 1386). But 2 months after the policy went into effect, most researchers seem to be ignoring it.

As of 2 July, NIH's PubMed Central had received only about 300 papers, a mere 3% of the 11,000 expected if all NIH grantees complied. Two-thirds of authors said NIH could post their paper immediately upon publication, and the rest asked for a delay.

Timothy Hays of NIH's extramural research office says the figure is "not surprising" because many grantees are waiting for their institutions to tell them how to respond to the new policy and for guidance from journals. But Sharon F. Terry, president of the Genetic Alliance, says it may be time for NIH to rethink things. "If we were … investing in a new business, and we saw early performance returns at the rate of 3%, we would not wait to reexamine our strategy," she says.

Voices in the Brain

When people with schizophrenia have auditory hallucinations, the voices they hear in 70% of cases are male, regardless of the sex of the patient. Now scientists in Britain say that's because female tones are more complicated for the brain to create.

They exposed 12 males to voices of both sexes while scanning their brains. The images indicated that female voices activated the auditory cortex more intensely than did male voices. Women have shorter vocal cords that produce a more complex range of sound frequencies, explains Michael Hunter, a psychiatrist and cognitive neuroscientist at the University of Sheffield, U.K. Hunter says that the male voices activated the "mind's eye"—part of the visual cortex in the back of the brain—probably because the male subjects were comparing the voices to their own. The lab is currently conducting the study with female subjects.

Although facial expressions have been studied a great deal, voices—which are like "auditory faces"—have gotten much less attention, says Pascal Belin, a neuroscientist at the University of Montreal in Canada. Hunter, whose paper is in press at the journal *NeuroImage*, says that if hallucinations affect the brain the same way as real voices, knowing that different types activate different brain areas could lead to targeted drug treatments to reduce spontaneous brain activity in schizophrenia.

Architecture for the South

New station will be able to ski out of danger.

A string of buildings reminiscent of a caterpillar on skis has won a design competition for the new Halley VI science station in Antarctica.

The British Antarctic Survey in Cambridge held a contest for eco-friendly designs that could withstand the extreme conditions at the Waddell Sea: 145-kilometer-per-hour winds, an average temperature of –30°C, and three sunless months a year. The new station also had to be mobile to avoid the fate of the existing one, which is on an ice shelf that is moving toward the sea at about 400 meters a year and that may calve off in the next decade.

The winner, from Faber Maunsell and Hugh Broughton Architects, features two wings joined by a large recreational center. Amenities include a climbing wall, hydroponics for growing salad greens, panoramic windows, and quarters for 52 people. The modules are easy to reconfigure, the designers say, and the interior has "strong, cheerful colors carefully selected with the help of a color psychologist" to keep away the polar blues. The retractable legs can step up to stay on top of new-fallen snow and are fitted with skis so the station can be towed away from the sea.

Construction on the new station, designed to last 20 years, should start in January 2007.

CREDITS (TOP TO BOTTOM): AP PHOTO/KEVIN P. CASEY; FABER MAUNSELL AND HUGH BROUGHTON ARCHITECTS

Edited by Yudhijit Bhattacharjee

JOBS

Corporate tussle. Seven years after founding Microsoft's research facility in Beijing, Kai-Fu Lee left the company earlier this month and announced that he would help Google launch a new lab in China. But first, the 43-year-old computer scientist must fend off a suit brought by his former employer, which claims that he's breaking a 1-year

no-competition clause in his contract.

"As a senior executive, Dr. Lee has direct knowledge of Microsoft's trade secrets concerning search technologies and China business strategies," says a spokesperson for Microsoft, which filed the suit in King County, Washington, Superior Court within hours of Google's 19 July announcement. Google says it will "defend vigorously" against Microsoft's "meritless" claims.

The Taiwan-born Lee, who more recently has directed the development of Microsoft's Internet search technology, says he accepted Google's offer in part for the opportunity to return to China. The new lab is expected to open this fall, although Google has yet to announce its location or number of employees.

DEATHS

A soaring idea. Paleontologist John Ostrom of Yale University, whose ideas helped kindle a "dinosaur renaissance," died 16 July of complications from Alzheimer's disease. He was 77.

In 1964, Ostrom discovered a 3-meter-long dinosaur that he named *Deinonychus* for "terrible claws." Its predatory prowess led Ostrom to propose the controversial idea that *Deinonychus* and other dinosaurs were warm-blooded. He later argued that birds had evolved from advanced, predatory dinosaurs. Initially greeted with skepticism, the view has since been widely accepted.

Ostrom's conception of dinosaurs as intelligent, active, and agile creatures stimulated much research, says Timothy Rowe of the University of Texas, Austin: "I don't think anyone else has had as broad an impact on the community."

Neuroscience alliance. A British neuroscientist is leading an initiative to build new partnerships between brain researchers in London and Paris.

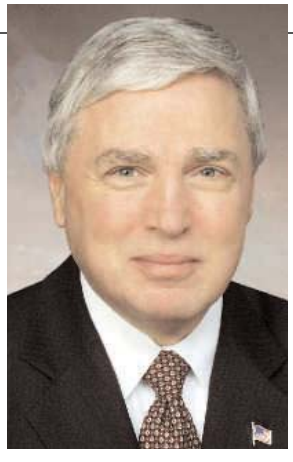
Richard Frackowiak says the old ideal of a European community of scholars prompted him to propose a research alliance between his institution, University College London (UCL), and the Ecole Normale Supérieure (ENS) and Université Pierre and Marie Curie, both in Paris. Under the partnership, inked earlier this month, the three institutions will conduct joint seminars and research projects and offer a joint master's degree allowing students to spend 1 year in each city. Frackowiak, 55, will serve as director of the department of cognitive studies at ENS in addition to his duties at UCL.

"There's a great thirst for more intensive collaboration across the channel," says Frackowiak, who's fluent in French.

MONEY MATTERS

Buying time. After stunning researchers 2 years ago by saying he hoped to end cancer deaths in the United States by 2015, National Cancer Institute Director Andrew von Eschenbach (right) now suggests it can be done even sooner. This month, in a response to Senator Arlen Specter (R–PA), chair of the Senate Appropriations subcommittee that funds NIH, von Eschenbach says his institute could meet its target of "eliminating suffering and death from cancer" by 2010 if its nearly $5 billion annual budget were boosted by $4.2 billion over 5 years.

The statement to Specter, who is battling Hodgkins lymphoma, warns that 2010 "may not be fully achievable," but that the money would help in "narrowing the gap." The boost would go largely to advanced technologies and infrastructure for clinical trials.



Got any tips for this page? E-mail people@aaas.org

CREDITS (TOP TO BOTTOM): YALE UNIVERSITY; MICROSOFT; NATIONAL CANCER INSTITUTE

LETTERS

Sound Advice or Just Plain Absurd?

THE LETTER "KEEPING AN OPEN MIND?" BY G. Anderson (22 Apr., p. 495) advises scientists to accept as "good advice" the Georgia textbook sticker on skepticism toward evolution. The sticker cited with approval reads in part that "Evolution is a theory, not a fact… This material should be approached with an open mind, studied carefully and critically considered." Skeptical thinking is an essential element in the scientific enterprise. But it is absurd to ignore the context of this sticker, which is aimed not at endorsing skeptical inquiry generally, but to advance a particular religious agenda. I doubt that the proponents of these stickers would support placing a sticker in the bibles, prayer-books, and hymnbooks used by churchgoers, asking them to approach their deism with skepticism.

DAVID JOHNS
Division of Political Science, School of Government, Portland State University, Post Office Box 751, Portland, OR 97207, USA. E-mail: johnsd@pdx.edu

A European Perspective on ID

IT IS A STRANGE EXPERIENCE FOR A EUROPEAN biologist to read about the growing support in the United States for so-called "intelligent design," the current name for good old unintelligent creationism. Strangest of all, though, are the recent activities of the Kansas Board of Education (Y. Bhattacharjee, "Kansas gears up for another battle over teaching evolution," News Focus, 29 Apr., p. 627; E. Reynolds, "A cry for help from Kansas," Letters, 29 Apr., p. 631). The Kansas Board's proposal to "[change] the definition of science" is unheard of in a western democracy, although similar activities have been common in dictatorships. In Nazi Germany, relativity was considered "Jewish science" and therefore unacceptable, while in the Soviet Union, modern genetics was rejected as unmarxist in favor of the ravings of the charlatan Lysenko. Is this the way the good citizens of Kansas (and the many other states where similar initiatives are seen) want to go?

Obviously, there must be a profound ignorance of science and the scientific method among the U.S. public for such a thing to happen (an ignorance that intelligent design supporters evidently hope to perpetuate), and for this, scientists must be held responsible. There is too much looking down at colleagues who engage the public through popular science, such as the late Carl Sagan (*1*). All scientists, not just biologists, should realize that an attack on the very roots of science concerns every one of them, and accordingly, they should do their utmost to counteract it by actively participating in the debate.

> "[T]here must be a profound ignorance of science and the scientific method among the U.S. public for such a thing to happen..."
>
> –FJERDINGSTAD

EJNAR J. FJERDINGSTAD*
Montauroux, France.
*Retired Professor of Anatomy, University of Aarhus, Denmark.

References
1. S. J. Gould, *Science* **275**, 599 (1997).

Getting the Right Info out to the Public

J. COUZIN'S ARTICLE "A HEAVYWEIGHT BATTLE over CDC's obesity forecasts" (6 May, p. 770) about the controversy over the Centers for Disease Control and Prevention's estimates of obesity-related deaths points out a major problem in public health: the often conflicting information that seems to emanate to the public from health-related scientific studies such as these. Aside from differences in study design and analysis of data, a big part of the problem is the premature proliferation in the popular press of health-related public messages based on these studies. For example, *The New York Times* gave front-page coverage to the latest findings under the misleading title "Some extra heft may be helpful, new study says" (*1*), increasing the likelihood that the results will be misinterpreted by many lay people who know nothing about statistics or epidemiology. Such studies would generate far less confusion if they were just left in the scientific literature until firm conclusions and recommendations were established.

What the public primarily needs to know about obesity was discussed at a conference at the New York Academy of Medicine in June 2004, namely, that obesity has not only reached epidemic levels in the United States but is actually now a pandemic, with increases being observed even in Asian countries like China, Thailand, and South Korea. There are many studies relating being overweight and obese to an increased risk for major systemic disease, like heart disease, hypertension, and diabetes. This needs to be emphasized much, much more than a tweaking of the numbers that suggests that being overweight with a BMI of 25 to 30 slightly reduces mortality risk.

JERRY RAPP
Department of Biological Sciences, State University of New York, 33 West 42nd Street, New York, NY 10036, USA.

Reference
1. G. Kolata, *N.Y. Times*, 20 Apr. 2005, p. A1.

Treating Medieval Manuscripts as Fossils

IN TREATING VELLUM MANUSCRIPTS AS FOSSILS, J. L. Cisne concludes that we possess a large portion of significant manuscripts that circulated in early medieval times ("How science survived: Medieval manuscripts' 'demography' and classic texts' extinction," Reports, 25 Feb., p. 1305). The result is not surprising. Rather, we would be surprised if a substantial proportion of canonical texts did not survive; noncanonical texts were those texts that were not as highly studied, copied, or preserved against destruction. Cisne's results hold promise for "fossilized" societies (see the accompanying Perspective "'How science survived'—medieval manuscripts as fossils," S. L. Gilman, F. L. Glaze, 25 Feb., p. 1208), where new knowledge neither infiltrates nor incubates. The observation that many canonical texts survive in more recently extinct societies, however, could be explained by a cultural version of paleobiology's "Pull of the Recent" (*1*), whereby data farther back in time are less well represented in the documentary (and fossil) record than younger ones. Although Cisne effectively demonstrates why historians tell us that the past is overdetermined [i.e., multiple lines of evidence converging on the same thesis (*2*)], his methods reflect nonphylogenetic

Letters to the Editor

Letters (~300 words) discuss material published in *Science* in the previous 6 months or issues of general interest. They can be submitted through the Web (www.submit2science.org) or by regular mail (1200 New York Ave., NW, Washington, DC 20005, USA). Letters are not acknowledged upon receipt, nor are authors generally consulted before publication. Whether published in full or in part, letters are subject to editing for clarity and space.

thinking about cultural evolution, an approach that has been questioned (*3*, *4*).

More importantly, books (or portions thereof) are not fossils. Unlike organisms, manuscripts have no definite life-span and books lack specific generation times and can be replicated rapidly or after centuries of neglect; consequently, replication confounds chronology and genealogy. Furthermore, just as with organismal "living fossils," we cannot assume that surviving medieval manuscripts are ancestral versions of more recent editions (*5*).

NICHOLAS D. PYENSON[1] AND LEWIS PYENSON[2]
[1]Department of Integrative Biology and Museum of Paleontology, University of California, Berkeley, CA 94720, USA. E-mail: pyenson@berkeley.edu. [2]Center for Louisiana Studies, University of Louisiana at Lafayette, Lafayette, LA 70504–0831, USA.

References
1. D. Jablonski *et al.*, *Science* **300**, 1133 (2003).
2. S. J. Gould, *Am. Sci.* **74**, 60 (1986).
3. R. D. Gray, Q. D. Atkinson, *Nature* **426**, 435 (2003).
4. R. Mace, C. J. Holden, *Trends Ecol. Evol.* **20**, 116 (2005).
5. M. D. Crisp, L. G. Cook, *Trends Ecol. Evol.* **20**, 122 (2005).

J. L. CISNE'S REPORT "HOW SCIENCE SURVIVED: medieval manuscripts' 'demography' and classic texts' extinction" (25 Feb., p. 1305) presents an unorthodox approach. Reasonable assumptions, applied in a geological model, do not seem equally applicable to the survival of medieval manuscripts, as discussed in the accompanying Perspective "'How science survived'—medieval manuscripts as fossils" (S. L. Gilman, F. L. Glaze, 25 Feb., p. 1208). Cisne's model is quite restrictive; it postulates a monotonous birthrate of manuscripts in time and assumes a finite and uniform loss rate, which even reduces to zero after the 15th/16th century. For medieval manuscripts, there is information that contradicts these model conditions. The manufacture of books during the Middle Ages was, as now, a question of continuously changing preferences. For instance, the ratio (signalizing the relative prevalence) of classical Greek and Roman texts to contemporary patristic texts by Christian church fathers changed more than a factor of 16 between the seventh and the ninth century, indicating large changes in "birthrates" for those classes of manuscripts (*1*). Geometric mean loss rates per century for medieval libraries in Great Britain varied from –22% for the 12th century to –40% for the 15th, as can be calculated with information presented in (*2*). These nonuniform medieval loss rates of manuscripts were followed by higher postmedieval losses (*1*). Unlike a collection of fossils, a distribution of manuscripts is not frozen in time; it continues to decay (*3*). Furthermore the chance of ultimate destruction, $(\mu/\lambda)^{M0}$, is presented as independent of the date of manufacture, contrary to what is observed. If Cisne had inserted any of the other values from Table 1 for μ/λ, he would have found considerably smaller destruction. Thus, I would not recommend this geological approach for medieval manuscripts.

ELTJO BURINGH
Utrechtseweg 90, 6866 CN Heelsum, the Netherlands.

References
1. E. Buringh, *Two Millennia of Manuscripts, a Study in Global Distributions of Manuscripts from 1 to 1900 AD* (Brill, Leiden, Netherlands, in press).
2. N. R. Ker, *Medieval Libraries of Great Britain* (Royal Historical Society, London, 1964).
3. M. Mostert, *The Library of Fleury, a Provisional List of Manuscripts* (Verloren, Hilversum, Netherlands, 1989).

Response

PYENSON AND PYENSON PERPETUATE A MIS-understanding that seems to have begun with the Perspective by S. L. Gilman and F. E. Glaze ("'How science survived'—medieval manuscripts as fossils," 25 Feb., p. 1208). The birth-and-death and logistic models have been successfully applied to a wide range of systems that can be conceptualized as a statistical population of particles, for instance, unstable atomic nuclei in an nuclear warhead or reactor, bacteria in a

growth medium, or lemmings on an arctic island (*1*, *2*). Contrary to what the Pyensons suggest, it makes no particular difference what the particles are or whether they have a circumscribed life-span, like the lemmings, or do not, like nuclei and bacteria. What counts is how well a model's predictions fit the data, which in the case of the logistic model and Bede's four particularly favorable manuscripts seems quite well enough.

The system in question is "the manuscripts of a text" without regard to time or place. Gilman and Glaze failed to distinguish between "text" as a set of words and "text" as a bound set of pages. They identified "the population of medieval scientific manuscripts [of many texts]" and "the population of texts [and translations thereof]" as systems equivalent to "the manuscripts of a text." They then immediately objected (quite rightly) that the model should not apply particularly well to systems to which they incorrectly conceived it should, pointing out that their "population of texts" is not a closed system because new texts (and translations thereof) were introduced from outside medieval Europe.

The Pyensons carry this misunderstanding a step further. The "Pull of the Recent," as originally conceptualized by Raup (*3*), applies to taxa (read: texts), not individuals (read: manuscripts). The phenomenon of textual stability (read: evolution) was well known to textual scholars long before Raup's time (*4*). Except perhaps for small fragments and short excerpts, identifying manuscripts as belonging to one or another text is not a problem, even in cases like the *Agrimensores* (*5*), in which the text was repeatedly and substantially updated to track changes in the subject matter (*5*, *6*).

It is unfortunate Buringh came away with the impression that the birth-and-death model or the logistic model is supposed to apply to manuscript populations in general. I pointed out in the Supplementary Online Material that particularly favorable "[c]ases like these four [Bede texts] may be more the exception than the rule" and that "[the case] of Bede's best-known work, *Historia Ecclesiastica Gentis Anglorum*…, may be more typical" in that its manuscript tradition is known to violate several of the model's assumptions. Few would deny that the logistic model has been successfully applied to many biological populations that happen to have grown under field or laboratory conditions approximating those the model assumes, or that the model has been valuable in conceptualizing more complex cases to which the model does not strictly apply. Someday, perhaps, scholars and scientists will be studying really complicated manuscript traditions using population models as sophisticated as those now used in fisheries management or epidemiology, and all will be aware that the birth-and-death model only works in estimating a logistically growing population's likelihood of ultimate survival at the few-particle stage, and that ultimate extinction is certain for any limited population. My aim in writing was simply to take a first step in that direction. I expect to learn much from Buringh's forthcoming publication, and trust we both are headed in the right general direction.

JOHN L. CISNE
Department of Earth and Atmospheric Sciences, Cornell University, Ithaca, NY 14853, USA. E-mail: cisne@geology.cornell.edu

References

1. W. Feller, *An Introduction to Probability Theory and Its Applications*, vol. 1 (Wiley, New York, ed. 2, 1957), pp. 272–276, 305–306, 407–411.
2. W. Feller, *An Introduction to Probability Theory and Its Applications*, vol. 2 (Wiley, New York, ed. 2, 1970), pp. 25–26, 52–53, 337.
3. D. M. Raup, *Bull. Carnegie Mus. Nat. Hist.* **13**, 85 (1979).
4. S. L. Montgomery, *Science in Translation* (Univ. of Chicago Press, Chicago, 2000), pp. 281–286.
5. B. Campbell, *The Writings of the Roman Land Surveyors* (Society for the Promotion of Roman Studies, London, 2000).
6. M. D. Reeve, in *Texts and Transmission*, L. D. Reynolds, Ed. (Oxford Univ. Press, Oxford, 1983), pp. 1–6.

Human Hierarchies, Health, and IQ

R. M. SAPOLSKY'S REVIEW "THE INFLUENCE of social hierarchy on primate health" (29 Apr., p. 648) begins and ends with the conundrum that there is a graded, inverse association between low socioeconomic position (SEP) and important health outcomes in humans. The nicely described animal work on stress responses and social hierarchies forms the main portion of the piece. The application of these findings to humans is critical, yet, apart from some examples of the physiological responses to stress, there is no clear series of data-based findings to take us mechanistically, in human samples, from human social hierarchy, to psychosocial stressors, to stress-related physiological responses, to adverse health outcomes and mortality.

In humans, there is another factor and other possible mechanisms to consider. It is surprising that there was no mention of intelligence (IQ). Childhood IQ is moderately strongly correlated with adult socioeconomic position. Lower IQ is also associated with increased rates of all-cause mortality (*1*, *2*), cardiovascular disease (*2*–*4*), hypertension (*5*), contact with psychiatric services (*6*), and other negative health outcomes (*7*). These associations remain after controlling for socioeconomic position in early life. Stable population variation in IQ is perhaps more consistent with the highly graded socioeconomic position-health relation than are the shifting effects of small-group rank on psychosocial stress. The well-replicated, although relatively recent finding that lower childhood IQ is related to later morbidity (*7*) and mortality experience affords hypotheses about mechanisms linking cognitive resources to health differences. These hypotheses merit consideration alongside the psychosocial stress hypothesis (*8*, *9*).

IAN J. DEARY,[1]* DAVID BATTY,[2] LINDA S. GOTTFREDSON[3]

[1]Department of Psychology, University of Edinburgh, Edinburgh, EH8 9JZ, UK. [2]Medical Research Council Social and Public Health Sciences Unit, University of Glasgow, Glasgow, G12 8RZ, UK, and Department of Psychology, University of Edinburgh, Edinburgh, EH8 9JZ, UK. [3]School of Education, College of Human Services, Education, and Public Policy, University of Delaware, Newark, DE 19716, USA.

*To whom correspondence should be addressed. E-mail: I.Deary@ed.ac.uk

References

1. L. J. Whalley, I. J. Deary, *Br. Med. J.* **322**, 819 (2001).
2. C. L. Hart *et al.*, *Psychosom. Med.* **65**, 877 (2003).
3. C. L. Hart *et al.*, *Soc. Sci. Med.* **59**, 2131 (2004).
4. G. D. Batty, E. L. Mortensen, A.-M. Nybo Andersen, M. Osler, *Paediatr. Perinat. Epidemiol.*, in press.
5. J. M. Starr *et al.*, *J. Hypertens.* **22**, 893 (2004).
6. N. P. Walker, P. M. McConville, D. Hunter, I. J. Deary, L. J. Whalley, *Intelligence* **30**, 233 (2002).
7. G. D. Batty, I. J. Deary, *Br. Med. J.* **329**, 585 (2004).
8. L. Gottfredson, I. J. Deary, *Curr. Dir. Psychol. Sci.* **13**, 1 (2004).
9. L. S. Gottfredson, *J. Pers. Soc. Psychol.* **86**, 174 (2004).

Response

DEARY *ET AL.* RAISE TWO IMPORTANT POINTS, with which I agree. The first is that it is immensely difficult to carry out studies in humans that would uncover the series of steps linking social experience all the way down to the reductive biology of health and disease. Thus, the Letter nicely reiterates the rationale for the paper, namely, the usefulness of studies of nonhuman species.

Their second point is that IQ may be an important variable in understanding the health/socioeconomic status relationship. This is absolutely so and is likely to be relevant in a number of ways (e.g., having access to the most current information regarding health risk factors, being able to understand the pertinence of such factors, and so on).

ROBERT M. SAPOLSKY

Stanford University, Stanford, CA 94305–5020, USA, and Institute of Primate Research, National Museums of Kenya.

BIOTECHNOLOGY

A Comparative Look at Governing Science

Julian Kinderlerer

Knowledge of biology has long been applied to enhance human health and food availability. In recent decades, as our improved understanding of biological processes has provided new ways to intensify our use of biology, major advances have become increasingly common. The United Nations Conference on Environment and Development (the June 1992 "Earth Summit" at Rio de Janeiro, Brazil) proclaimed that "Humanity stands at a defining moment in history. We are confronted with a perpetuation of disparities between and within nations, a worsening of poverty, hunger, ill health and illiteracy, and the continuing deterioration of the ecosystems on which we depend for our well-being" (*1*). Then and since, there has been a widespread belief that modern biotechnology could provide solutions to many of the fundamental problems facing the world.

However, whether applied to humans, agriculture, or industrial purposes, the use of modern genetics has courted controversy. Almost as soon as people realized the possibilities of identifying genes and their functions within organisms or of moving genes between organisms, it was appreciated that there were implications that went beyond science. The manner in which different countries have addressed the issues arising from the attempts by scientists to use modern biotechnology has varied considerably. An examination of these different approaches may help us understand the cultural forces that underlie the way in which countries exploit the resources available to them.

In *Designs on Nature*, Sheila Jasanoff explores the ways in which genetics has been used in the last decades of the 20th century in three countries that have taken disparate approaches to the same problems: the United States, the United Kingdom, and Germany. Her analysis considers both "green" and "red" biotechnology. Green biotechnology covers applications in agriculture (such as engineering desirable traits into farm animals) and the environment (such as bioremediation). Red biotechnology comprises applications in biomedicine (such as diagnostic tests and genetically engineered therapeutic agents). As Jasanoff (a professor of science and public policy at Harvard University's John F. Kennedy School of Government) notes, the two strands involve "somewhat different scientific debates, ethical concerns, and political questions."

Designs on Nature
Science and Democracy in Europe and the United States
by Sheila Jasanoff
Princeton University Press, Princeton, NJ, 2005. 380 pp. $35.00, £22.95. ISBN 0-691-11811-6.

The book begins with a startling statement: "In mid-November 2001, Europe was forming in the oddest of places." Does Jasanoff mean a forging of a true European position on modern biotechnology, a new coming together of European thought in general following the cataclysms that had engulfed Europe in the middle of the 20th century, a revival of the concepts that had led to the formation of the European Union, or something more profound? I was not sure, because the prologue makes it clear that the different positions taken to the use of modern biotechnology within Europe (whether red or green) do not indicate a common philosophical position. The author's opening assertion made me read the book with care and fascination. Jasanoff devotes much of the text to first describing the historical context in which decisions about the use of biotechnology have been made in each of the three countries and then drawing conclusions about the basis for the differences that are manifest. This is particularly interesting in the European context for green biotechnology because the formal legal framework has, since 1990, been the same in the United Kingdom and Germany.

All three countries instituted some sort of legal framework to assure the safe use of the new bioengineering techniques that had been identified in the 1960s and 1970s. The initial framework in the United States was devised by the National Institutes of Health. Its guidelines were to be followed by all who received federal funding. They addressed both red and green biotechnology and were put in place in the late 1970s. In 1986, the U.S. Office of Science and Technology Policy published a "Coordinated Framework for Regulation of Biotechnology" (*2*), which explained its decision not to turn to specific legislation to regulate the products of modern biotechnology. The United States preferred to address the risks and benefits associated with a particular product regardless of the technology used to manufacture that product and therefore chose to rely on existing legislation and agencies to ensure that products introduced into the environment were safe. The coordinated strategy provided for product-specific regulation, an approach that was seen to offer the "opportunity for similar products to be treated similarly by particular regulatory agencies" (*2*). Most important, the system chosen in the United States stressed the primacy of "science-based" decision-making, in which policy and politics are divorced from the decision process.

The United Kingdom took a very different line. After starting with a voluntary system for researchers, it moved to a regulation-based system under the Health and Safety at Work Act of 1974 (*3*). Initially, these regulations only addressed the safety of those working with genetically modified (*4*) organisms (GMOs) or those who had legitimate reasons for entering GMO laboratories. In time, the remit was slowly widened to take into account the impact of such organisms on the environment. The trigger for regulation was specifically the process of genetic modification, rather than merely the characteristics of the product. Germany took an approach similar to that of the United Kingdom, with the process acting as the trigger for strict regulation to ensure safe use of the technology.

In 1990, the European Union enacted two directives for implementation by member countries: 90/219 addressed the contained use of genetically modified microorganisms, whereas 90/220 addressed the

CREDIT: PRESTON HUEY/*SCIENCE*

The reviewer is at the Sheffield Institute of Biotechnological Law and Ethics, University of Sheffield, 169-171 Northumberland Road, Sheffield, S10 1DF, UK. E-mail: j.kinderlerer@sheffield.ac.uk

noncommercial release of GMOs into the environment as well as the placement of GMO products on the market. Both directives required that the use of modern biotechnology trigger a regulatory process. Member countries interpreted these directives differently, and the differences may reflect their distinctive cultural or historical perspectives. Directive 90/220 contains provisions for the replacement of its requirements in circumstances where European legislation provides "for a specific environmental risk assessment similar to that laid down in this Directive." The provisions have subsequently been used to centralize the regulatory system for food and feed (in directive 2001/18 and regulation 1829/2003). Although the approval process requires a science-based risk assessment, the decision-making system is not divorced from either politics or policy-making.

The book is worth reading simply for Jasanoff's fascinating descriptions and explanations of the different interpretations and understandings of biotechnology regulation. The interpretations also provide an interesting perspective on the decisions for patenting higher life forms that have been made in each of the jurisdictions during the last 25 years.

Surprisingly absent from the book is any discussion of the protracted negotiations over an international treaty for green biotechnology. The United States signed but did not ratify the Convention on Biological Diversity (introduced at Rio de Janeiro in 1992) and hence has only been an observer during the negotiation of subsequent protocols. The Cartagena Protocol on Biosafety came into effect in 2003 and now has 120 members (including the European Union countries and China, but excluding such main producer countries as Argentina, Canada, and the United States). The protocol adopts the same trigger as the European Union chose in 1990. Critically, embedded in the protocol is the precautionary approach identified in the 1992 Rio declaration (*5*) and strongly resisted by the United States. The political and philosophical divisions that Jasanoff addresses were clearly identified in the negotiations for the treaty and text. Consideration of these treaty negotiations would probably have reinforced her analyses of green biotechnology.

Designs on Nature addresses red biotechnology in depth, considering the regulation (or absence of regulation) imposed by the three legal systems. The United Kingdom favors positions quite different from those taken by Germany and the United States. Its concept of the pre-embryo as simply a collection of cells (but which should be accorded some dignity) has provided the basis for permitting research on embryos and relatively liberal abortion law. Jasanoff discusses the clash between the use of embryonic stem cells or pre-implantation genetic diagnosis and a presumption that from the moment of conception there exists a person for whom the constitution provides protection. She offers an absorbing account of the course of the debate in the United States, where liberality in pre-implantation genetic analysis contrasts with the prohibition of federal funding for embryonic stem cell work.

One chapter is devoted to exploring how bioethics has contributed to differences in the regulation of biotechnology (in particular, cloning and stem cell research) among the three countries. Jasanoff reviews the governments' use of bioethics as an instrument of public policy and the efforts of nonstate actors (including industry and other nongovernmental organizations) to marshal bioethical arguments to support their goals.

Jasanoff offers her comparative analysis as a means to explain outcomes rather than prescribe policy. She argues that the differ-

ent responses to biotechnology in the United Kingdom, Germany, and the United States are each the result of "complex entanglements among knowledge, technical capability, politics, and culture." *Designs on Nature* should be read by all interested in the science or management of biotechnology, whether red or green, on both sides of the Atlantic.

References and Notes

1. *Agenda 21: Programme of Action for Sustainable Development* (United Nations Conference on Environment and Development, Rio de Janeiro, Brazil, 1992), chap. 16.
2. Office of Science and Technology Policy, *Fed. Reg.* **51**, 23302 (1986). (Available at www.epa.gov/fedrgstr/EPA-TOX/pre1994/ost86.pdf.)
3. The text of the act is available at www.healthandsafety.co.uk/haswa.htm.
4. The original wording was "manipulated."
5. Principle 15 of the declaration (www.un.org/documents/ga/conf151/aconf15126-1annex1.htm) states: "In order to protect the environment, the precautionary approach shall be widely applied by States according to their capabilities. Where there are threats of serious or irreversible damage, lack of full scientific certainty shall not be used as a reason for postponing cost-effective measures to prevent environmental degradation."

10.1126/science.1114030

PSYCHOLOGY

We're Not Fred or Wilma

Johan J. Bolhuis

Evolutionary psychology is a research paradigm that proposes that the human mind consists of cognitive modules that evolved in response to selection pressures faced by our Pleistocene predecessors. The discipline's short history has not lacked controversy—the late Stephen Jay Gould even suggested that evolutionary psychology does not qualify as science. Thus, the contribution to the debates on evolutionary psychology David Buller makes in *Adapting Minds* is important for two reasons.

First, Buller, a philosopher at Northern Illinois University, approaches the subject from the outside—not as a psychologist or evolutionary biologist. While on a semester's leave in London, he was struck by a television broadcast on human sexual behavior. The topic was interesting enough, but in addition Desmond Morris was on hand to explain with great confidence that our mating habits are a reflection of our evolutionary past. Buller, fascinated with such explanations of human behavior, wanted to find out more and thus had to come to grips with some biological essentials. As a result, readers can benefit from the author's exposé of his newly acquired insights into evolutionary biology, genetics, and cognitive psychology.

Second, Buller remains hopeful that an evolutionary approach to human psychology can be useful. He makes a helpful distinction between evolutionary psychology as a paradigm and as a broader field of research. (To avoid confusion, he consistently capitalizes the former.) As Buller is not averse to an evolutionary approach to human psychology, he is generally fair toward evolutionary psychologists. Consequently, he provides some fine matter-of-fact analyses of debates that are commonly conducted with a level of aggression that I imagine would have been very useful in disputes over scarce resources on prehistoric East African plains. For example, Buller demonstrates that some of Gould's criticisms of evolutionary psychology cannot be maintained. Nevertheless, Buller eventually concludes that the paradigm is not particularly well founded theoretically. One of its key claims is that "our modern skulls house stone-age minds"—that the human mind has not evolved significantly since the Pleistocene. Buller offers overwhelming evidence for the contrary conclusion. As he puts it, "There is no reason to think that contemporary humans are, like Fred and Wilma Flintstone, just Pleistocene hunter-gatherers struggling to survive and reproduce in evolutionarily novel suburban habitats."

Stone-agers in suburbia.

Adapting Minds
Evolutionary Psychology and the Persistent Quest for Human Nature
by David J. Buller
MIT Press, Cambridge, MA, 2005. 564 pp. $34.95, £22.95. ISBN 0-262-02579-5.

Adapting Minds' great strength is its author's willingness to give the paradigm's theory the benefit of the doubt. The bulk of the book consists of a thorough analysis of key data offered in support of the paradigm. Evolutionary psychologists argue that the human mind consists mainly of domain-specific modules, self-contained "minicomputers" that each process information of a certain kind. Perhaps the most famous of all is the "cheater detection module" proposed by Leda Cosmides and John Tooby. Through a thorough philosophical analysis conducted in collaboration with Valerie Gray Hardcastle, Buller demonstrates that the data provide no evidence for such a module. Instead, extant data are much more consistent with a role for the mind as a "general purpose problem solver." Buller concedes that there may be some degree of modularity of brain and mind, but he sees that as confined to "basic emotional adaptations" and sensory systems. This view is entirely consistent with that of Jerry Fodor, who first proposed the concept of modularity of the mind.

Buller devotes three chapters to the paradigm's interpretations of mate preferences, marriage and infidelity, and parenthood. In one, he focuses on Martin Daly and Margo Wilson's evidence for what they have called "discriminative parental solicitude." They provided data suggesting that children are much more likely to suffer abuse from stepparents than from their biological parents. Their findings are consistent with an evolutionary interpretation whereby parental investment is directed at increasing the chances of survival of one's own genes. Buller argues that Daly and Wilson's analysis is influenced by a reporting bias. He and Elliott Smith have analyzed a large dataset on child abuse in the United States, and they conclude that the evidence does not support the evolutionary psychology hypothesis.

The author's restraint and generous stance ensure that evolutionary psychologists have to take *Adaptive Minds* seriously. Buller is very critical of the paradigm, but it is hard not to be after all the theoretical arguments and empirical evidence against it. In his epilogue, he suggests that the paradigm does not suffer from one fundamental flaw but that it "makes little mistakes at nearly every theoretical and empirical turn." Buller ends on a hopeful note, suggesting that there has in fact been some progress toward an evolutionary understanding of the human mind. Although I do not share his optimism, I highly commend him for having written an outstanding book. It sets the standard for the continuing debates on evolutionary psychology.

10.1126/science.1115209

The reviewer is in the Behavioral Biology Group, Utrecht University, Post Office Box 80086, 3508 TB Utrecht, Netherlands. E-mail: j.j.bolhuis@bio.uu.nl

OCEANS

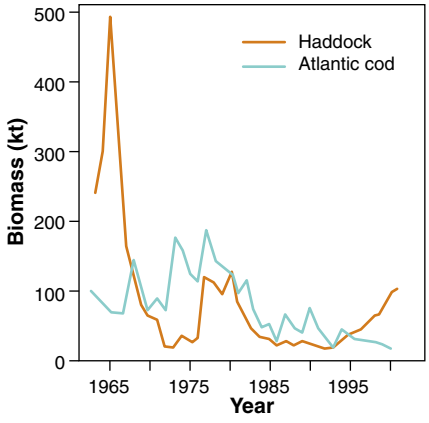
U.S. Ocean Fish Recovery: Staying the Course

Carl Safina,[1*] Andrew A. Rosenberg,[2] Ransom A. Myers,[3]
Terrance J. Quinn II,[4] Jeremy S. Collie[5*]

With many ocean fish populations at unprecedented lows and declining (*1*, *2*), management should now emphasize population rebuilding. The United States assumed leadership here with the rebuilding provisions of the Sustainable Fisheries Act of 1996. Unfortunately, attacks by some in both Congress and the courts would cut the heart out of the act and take policy backwards.

Currently the act mandates that federal fishery managers must adopt plans to end overfishing, and, within 10 years (unless biology dictates longer), rebuild depleted populations to levels that can support "maximum sustainable yield" (MSY). This recovery mandate is unique, and the United States now has numerous species whose incipient and ongoing population recoveries can be linked to management actions, most designed to meet these mandates. Retaining and strengthening these mandates is crucial. Attacking the recovery provisions tends to go against the long-term interest of the nation and its fishing and seafood communities and businesses.

Nonetheless, both the United States's mandate to end overfishing and its rebuilding time frame are under assault. Because rebuilding means that fishing mortality must first be reduced, commercial fishing interests, and certain members of Congress, are attacking the 10-year time frame as too rigid, aggressive, and arbitrary. In March, a federal court ruled, somewhat illogically, that managers could allow overfishing during a recovery plan's rebuilding period, as long as the population is rebuilt by the end of the period (*3*).



Depletion and renewal. Population trajectories of haddock and Atlantic cod on Georges Bank, off New England (kilotons).

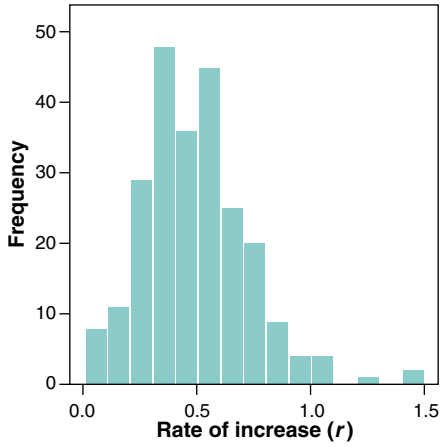
Recent legislation proposed in the House (HR 3645) and Senate [S 482, S 2066 (*4*)], and new circulating drafts, would change the mandate "end overfishing" to "address overfishing," and delay or even eliminate the rebuilding time frame.

NOAA's National Marine Fisheries Service, the agency responsible for implementing fisheries conservation and management legislation, has recently published proposed changes to the guidelines for applying overfishing and rebuilding standards in fishery management plans. The guidelines are used by the agency and the regional fisheries management councils set up under the Magnuson-Stevens Act when developing fishery rebuilding plans for overfished fisheries. The new proposals still call for rebuilding in as short a time as possible in principle. However, instead of a clear, unambiguous 10-year time frame for most stocks, the proposed rebuilding time frame is to be based on a theoretical rebuilding time under no fishing plus one mean generation time, defined as the average age of spawners for an unfished stock. The effect of the proposal may shorten some rebuilding windows, but in many cases is likely to result in a longer rebuilding schedule, particularly because the mean generation time for an unfished stock may be very much longer than that for an overfished stock with a highly truncated age distribution. The comment period on the proposed rule is open until 22 August 2005.

Evaluating the 10-Year Window

Both the U.S. Commission on Ocean Policy and the Pew Oceans Commission clearly emphasized last year that the nation's ocean resource policy must focus on rebuilding populations and ending overfishing (*5*). Ten years is a reasonable and beneficial rebuilding window. During drafting of the Sustainable Fisheries Act, several population dynamics experts pointed out that many depleted marine organisms were capable of rebuilding to target levels within about 5 years if fishing for them ceased. Drafters then looked at balancing the need for resource rebuilding with short-term concerns of managers and fishers. But the drafters also recognized that too long a rebuilding time frame would facilitate years of inaction, continued overfishing, and even increased catches, causing further population declines as has happened elsewhere (*6*). Ten years (twice the time the majority of populations require for rebuilding) was chosen to avoid Draconian mandates; to help ensure that managers actually commence rebuilding; to increase chances for success; and to minimize future ecological, social, and economic costs. This optimizing balance was deliberate and compassionate, not arbitrary.

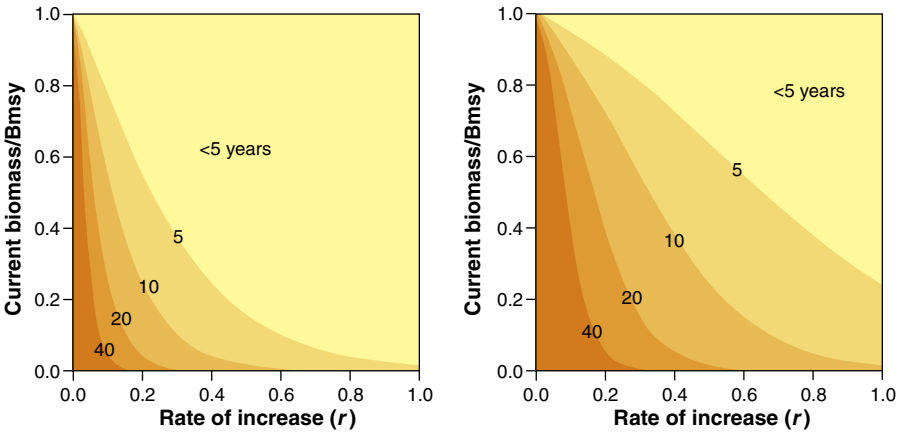
Atlantic black sea bass, scup, summer flounder, sea scallops, yellowtail flounder, and king mackerel are examples that owe their success to the fact that fishery managers acted early in the rebuilding window to reduce overfishing so as to hit projected targets within 10 years. All these species are



Distribution of intrinsic rates of increase (*r*) for 242 fish populations of commercial value (*25*, *26*).

[1]Marine Sciences Research Center, Stony Brook University, Stony Brook NY 11794, USA. [2]College of Life Sciences and Agriculture, University of New Hampshire, Durham, NH 03824, USA. [3]Biology Department, Dalhousie University, Halifax, Nova Scotia, Canada B3H 4J1. [4]Juneau Center, School of Fisheries and Ocean Sciences, University of Alaska Fairbanks, Juneau, AK 99801, USA. [5]Graduate School of Oceanography, University of Rhode Island, Narragansett, RI 02882, USA.

*Author for correspondence. E-mail: csafina@blueocean.org



Years required for rebuilding fish population. (Left) Rebuilding times with no fishing, assuming a Graham-Schaefer model [(*27*), Eq. (2.9)]. Rebuilding time depends only on the intrinsic rate of increase r, fishing mortality F, and the biomass at the onset of rebuilding B_0, expressed as a proportion of the biomass needed to produce MSY B_{msy}: $t = [1/(r–F)\ \ln\{[(B_0/B_1)^{-1}\ 2(1–F/r)–1]/[2(1–F/r)–1]$. Most combinations have rebuilding times less than 5 to 10 years (yellow, orange). (Right) Rebuilding times with fishing mortality at 80% of the rate associated with MSY.

significantly more abundant now than a decade ago, so that fishing can increase.

In our opinion, this approach fails when management delays resulting from political pressure allow continued overfishing; the rebuilding clock keeps ticking and populations decline further. This failure to act early, necessitating deeper fishery cuts to rebuild populations within the time limit, has prompted critics to argue that a longer rebuilding window will be necessary (*7*).

It doesn't work that way. Human predilection for inaction necessitated the rebuilding time frame in the first place, and deadlines will be needed unless and until human nature changes. New England cod and haddock populations—classic overfishing examples (*8*)—exemplify the contrast (see figure, page 707, top). Fishing pressure on haddock was abruptly reduced, but managers phased in cod fishing reductions slowly. Haddock rebounded quickly, now supporting lucrative fishing. Cod have scarcely increased, and restrictions are affecting and will affect the industry years later, when recovery could be nearing completion.

Risks of Prolonging Overfishing

Delaying rebuilding puts ecosystem components at risk (*9–11*). Gulf of Maine cod, for example, are missing from nearly half their coastal spawning grounds of 50 to 70 years ago, apparently because many small local populations are now extinct (*12*). Atlantic pollock have similarly disappeared off Block Island (*13*), Atlantic bluefin tuna are gone from large parts of their range (*14*), and other extirpations have likely gone unnoticed. Overfishing truncates a population's size and age distribution, lowers genetic diversity, and suppresses reproductive and recovery capacity (*15*, *16*). Biocomplexity is critical for resilience, and population persistence will likely require adaptation to changing conditions (*17, 18*). Warming is already challenging heavily exploited Atlantic salmon, North Sea cod, Long Island Sound lobsters, and others (*19–21*). Prolonged depletion also incurs ecosystem cascades; e.g., blue crab overfishing has contributed to mass salt-marsh grass die-off, because predatory crabs normally suppress herbivorous snails (*22*). In sum, the longer managers allow overfishing, the more depletion undermines subpopulations' diversity, resilience, and adaptability; risks ecosystem structure and functioning; reduces chances for eventual recovery; and raises social and economic costs.

Rebuilding Imperatives

The great majority of marine fish populations have intrinsic increase potential (see page 707, bottom) that, absent fishing, would rebuild them to target levels within 10 years (see figure above, left). Fishing at half the fishing mortality rate associated with MSY would only moderately delay rebuilding. However, fishing depleted populations at 80% of the fishing mortality for MSY [as suggested in S2066 (*4*)] would greatly delay rebuilding (see figure above, right).

Overfishing must be quickly prohibited, and management must be required to keep depleted populations continually increasing. The North Pacific Fishery Management Council, widely acknowledged for managing one of the most stable, high-volume, and lucrative fisheries in the world, automatically reduces fishing mortality as a population declines below its target level (*23*), i.e., more fishing when there are more fish, less fishing when there are fewer fish. It need surprise no one that this sensible approach generally maintains robust exploited populations, high levels of fishing activity, and big money. Such a sensible approach should be universally required and is well suited for populations biologically incapable of rebuilding within 10 years.

Maximizing economic, social, and ecological benefits requires ending, not tolerating, the damages of costly overfishing. The United States must retain its leadership with its timed, mandated approach to rebuilding depleted fishery populations. A required time frame is most desirable for starting and assessing rebuilding progress. For most species a 10-year rebuilding window accomplishes these objectives and should be retained. These minimum recommendations do not address the need to reduce unwanted bycatch or to maintain ample quantities of prey, rare species, high-quality and refuge habitats, and other ecosystem concerns.

References and Notes

1. D. Pauly *et al.*, *Science* **302**, 1359 (2003).
2. R. A. Myers, B. Worm, *Nature* **423**, 280 (2003).
3 *Oceana Inc.* et al. vs. *D. E. Evans* et al., U.S. Dist. LEXIS 3959 (2005); available at New England groundfish 2005, Huvelle ruling 7-6-05.pdf
4 These bills were all introduced in the 108th Congress, which ended last fall. None of them have been reintroduced and, therefore, are not under consideration by Congress.
5. C. Safina, S. Chasis, *Issues Sci. Technol.* **21**, 37 (2004).
6. C. Safina, *Conserv. Biol.* **7**, 229 (1993).
7. Magnuson-Stevens Act Provisions: National Standard Guidelines, Proposed Rule; see comments section in the Proposed Rule for the National Standard Guidelines (*24*).
8. S. A. Murawski *et al.*, *NOAA Tech. Mem.* NMFS-F/SPO-41, 71 (1999).
9. R. S. Steneck, *Trends Ecol. Evol.* **13**, 429 (1998).
10. J. A. Hutchings, *Nature* **406**, 882 (2000).
11. J. B. C. Jackson *et al.*, *Science* **293**, 629 (2001).
12. E. P. Ames, *Fisheries* **29**, 10 (2004).
13. D. Preble, C. Safina, in *The Good In Nature and Humanity* (Island Press, Washington, DC, 2002), p. 175.
14. M. P. Sissenwine *et al.*, *Trans. Am. Fish. Soc.* **127**, 838 (1998).
15. D. O. Conover, S. B. Munch, *Science* **297**, 94 (2002).
16. S. Berkeley *et al.*, *Fisheries* **29**, 23 (2004).
17. L. Hauser *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **99**, 11742 (2002).
18. R. Hilborn *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **100**, 6564 (2003).
19. G. Beaugrand *et al.*, *Nature* **426**, 661 (2004).
20. M. Edwards, A. J. Richardson, *Nature* **430**, 881 (2004).
21. K. D. Friedland *et al.*, *Can. J. Fish. Aquat. Sci.* **60**, 563 (2003).
22. B. R. Silliman, M. D. Bertness, *Proc. Natl. Acad. Sci. U.S.A.* **99**, 10500 (2002).
23. D. Goodman *et al.*, "Scientific review of the harvest strategy currently used in the BSAI and GOA groundfish fishery management plans"; www.fakr.noaa.gov/npfmc/misc_pub/f40review1102.pdf (2002).
24. *Fed. Regist.* **70** (119), 36239 (22 June 2005).
25. R. A. Myers *et al.*, *Can. J. Fish. Aquat. Sci.* **56**, 2404 (1999).
26. R. A. Myers *et al.*, *Fish. Bull.* **95**, 762 (1997).
27. T. J. Quinn II, R. B. Deriso, *Quantitative Fish Dynamics* (Oxford Univ. Press, New York, 1999).
28. We thank M. Bowman, S. Chasis, L. Crocket, J. Johnson A. Ottensmeyer, E. Pikitch, S. Pimm, and R. Wagner for information, comments, and help with the figure.

10.1126/science.113725

MICROBIOLOGY

Translocation of Anthrax Toxin: Lord of the Rings

Gunnar von Heijne

Eukaryotic cells are endowed with exquisite machineries for moving proteins across their lipid membranes and out of the cell. But just as every coin has two sides, microorganisms have evolved equally ingenious machineries that allow them to ship toxic proteins into target cells. Although these machineries seem to have evolved largely independently of one another, they have converged onto a common basic principle: to thread proteins across membranes as linear, unfolded polypeptide chains rather than as more unwieldy, tightly folded globular structures.

Enhanced online at www.sciencemag.org/cgi/content/full/309/5735/709

Even when unfolded, a polypeptide cannot by itself penetrate through a lipid membrane. It needs some kind of proteinaceous channel to provide a passageway. On page 777 of this issue, Krantz *et al*. (*1*) provide new insight into how such a channel—the anthrax toxin pore—works.

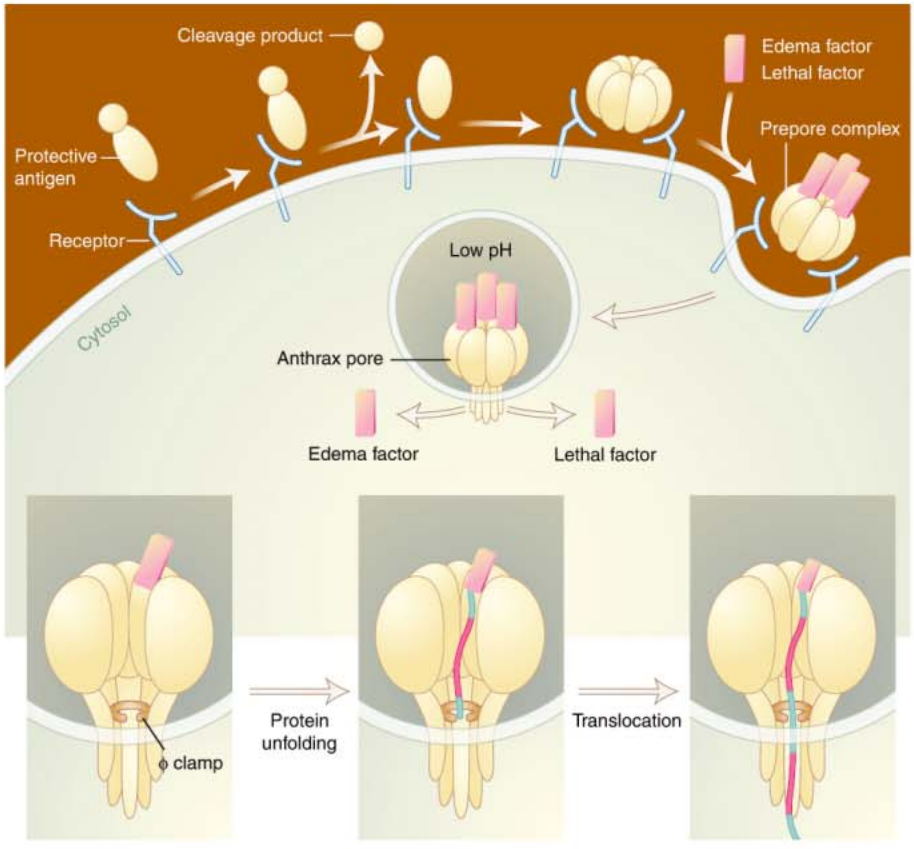
Anthrax is caused by the bacterium *Bacillus anthracis* and normally spreads to humans via infected animals or contaminated animal products. Inhalation of bacterial spores is particularly dangerous, with a mortality rate of nearly 100% if no treatment is given. Fortunately, anthrax is a rare disease, although it is regarded as a threat in the bioterrorism arena.

Anthrax toxin is secreted by the bacterium in the form of three distinct proteins (*2*) (see the figure). The pore-forming protective antigen protein first binds to a receptor on the surface of the target cell. It is activated by a proteolytic cleavage event and assembles into a heptameric prepore complex. The lethal factor and edema factor proteins then bind to the prepore complex, whereupon the whole assembly is taken up by the target cell and delivered into an acidic intracellular compartment. The low-pH environment triggers a conformational change in the prepore that leads to formation of the toxin pore proper. The low pH simultaneously causes a partial unfolding of the lethal and edema factor proteins, priming them for transport through the pore. Finally, each factor is threaded through the pore in a poorly understood process driven by the electrochemical potential across the membrane.

The pore itself has the overall shape of a mushroom with its stem penetrating the membrane of the target cell. The current model of the stem is that of a 14-stranded β barrel, 15 Å in diameter, with a water-filled pore running down its center (*3*). This is the correct diameter to allow the passage of an unfolded polypeptide, but how can the pore catalyze the complete unfolding of the toxin proteins to promote their transport into the cell?

To address this question, Krantz *et al*. mutated amino acids thought to line the pore to cysteine, and then modified these residues with a small organic reagent. Modification of two neighboring residues in the pore—Phe^{427} and Ser^{429}—blocked ion conductance, suggesting that they define the most narrow part of the pore. Further studies of Phe^{427} by electron paramagnetic resonance showed that the seven copies of this residue—one from each of the seven subunits—move close to each other during the prepore-to-pore transition and thus presumably form an aromatic ring or "ϕ-clamp" that constricts the pore.

The authors then made a very surprising observation. The pore's ion conductance increases, as expected, when Phe^{427} is mutated to smaller residues such as Ala or Ser. In contrast, the translocation rate through the pore of a fragment from the lethal factor protein decreases to an undetectable level when Phe^{427} is replaced by Ala. The Ala^{427} mutation consequently inactivates the toxin. Paradoxically, removal of the ϕ-clamp makes the pore a less efficient translocator, which strongly suggests that the ϕ-clamp is



CREDIT: PRESTON HUEY/*SCIENCE*

How anthrax infects cells. Insets show how hydrophobic segments (blue) in the unfolded toxin protein (edema or letal factor) bind in succession to an aromatic ring, or ϕ clamp, promoting translocation into the host cell cytoplasm.

The author is in the Department of Biochemistry and Biophysics, Stockholm University, SE-10691 Stockholm, Sweden. E-mail: gunnar@dbb.su.se

not simply constricting the pore but has an active role in the translocation process.

What, then, might be the function of the ϕ-clamp? To probe its substrate-binding properties, the authors tested the inhibitory effects on ion conductance of a panoply of small blocking agents such as tetrabutylammonium or tetraphenylphosphonium. They concluded that the ϕ-clamp binds these agents by nonspecific hydrophobic as well as aromatic π-π and cation-π interactions. This led them to the idea that the role of the ϕ-clamp is to grab successive hydrophobic segments in the lethal and edema factor proteins as each chain is pulled through the pore. In essence, they propose a "Brownian ratchet" model where transient unfolding of the protein exposes hydrophobic segments that bind to the ϕ-clamp, preventing refolding and facilitating the conversion to a translocation-competent form.

There is a striking analogy between the ϕ-clamp structure proposed by Krantz *et al.* and the so-called hydrophobic gasket found in the Sec61 translocon that mediates protein translocation across the inner membrane of bacteria and the endoplasmic reticulum membrane of eukaryotes (*4*). In the Sec61 channel, a ring of hydrophobic Ile residues is thought to provide a flexible seal around the translocating polypeptide, preventing ion leakage through the membrane. At the same time, the Ile ring is expected to bind to hydrophobic segments in the polypeptide, perhaps shunting very hydrophobic transmembrane helices into the surrounding lipid membrane. Another comparable case is the GroEL/ES chaperonin in which hydrophobic residues project into a central cavity. This configuration is thought to unfold entrapped, misfolded proteins by pulling on hydrophobic residues exposed on their surface (*5*). And recently, it was proposed that movable loops protruding into the central channel of the ATP-driven ClpA protease push unfolded substrates toward their destruction (*6*).

These likely represent only a few examples of how cells manipulate unfolded proteins. By exploiting the most basic characteristic of the unfolded state—the exposure of hydrophobic residues—cells have microengineered sophisticated molecular machines to push and pull proteins, delivering them to their final destinations.

References

1. B. A. Krantz *et al.*, *Science* **309**, 777 (2005).
2. R. J. Collier, J. A. T. Young, *Annu. Rev. Cell Dev. Biol.* **19**, 45 (2003).
3. B. A. Krantz, A. D. Trivedi, K. Cunningham, K. A. Christensen, R. J. Collier, *J. Mol. Biol.* **344**, 739 (2004).
4. B. van den Berg *et al.*, *Nature* **427**, 36 (2004).
5. W. A. Fenton, A. L. Horwich, *Q. Rev. Biophys.* **36**, 229 (2003).
6. J. Hinnerwisch, W. A. Fenton, K. J. Furtak, G. W. Farr, A. L. Horwich, *Cell* **121**, 1029 (2005).

10.1126/science.1116630

PHYSICS

Logical Spectroscopy

Ekkehard Peik

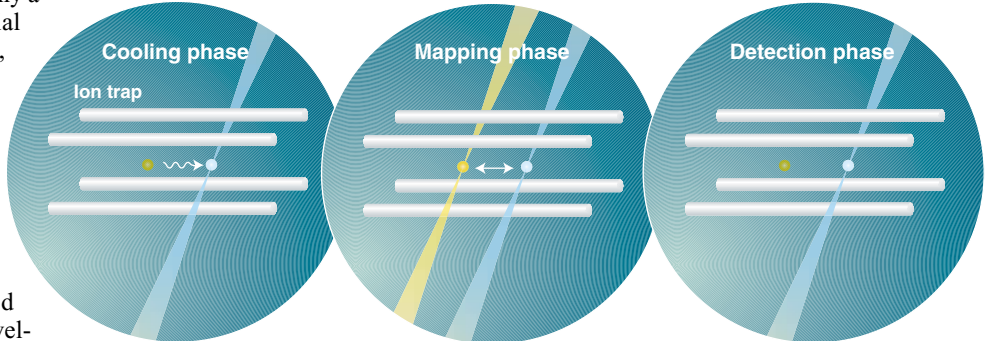
Precise knowledge of the frequencies of emission lines produced by quantum state transitions in atoms is essential for tests of fundamental physics as well as for the development of better clocks and measurement standards. The most precisely known atomic resonance frequencies in the optical spectral range are currently those of transitions in the positive ions of strontium, mercury, and ytterbium (*1*–*3*). At first thought, these species may not appear to be models of simplicity, nor are they treated very prominently in classical spectroscopy textbooks. They provide, however, a combination of properties that make them well suited for the application of laser spectroscopy methods that permit the highest precision. Unfortunately, only a limited number of ions have these special properties. On page 749 of this issue, Schmidt *et al.* (*4*) demonstrate a technique that dramatically widens the range of atoms or molecules that are amenable to this kind of precision spectroscopy. Key ingredients of the method have been developed in the context of a different research field—quantum information processing. Now, these quantum logic tools may find another important application in the development of optical clocks.

The author is in the Time and Frequency Department, Physikalisch-Technische Bundesanstalt, 38116 Braunschweig, Germany. E-mail: ekkehard.peik@ptb.de

The seminal ideas for laser spectroscopy of trapped ions were put forward by Dehmelt more than 30 years ago (*5*). The methods were developed and perfected by several groups and became highly successful (*2*, *3*). Precision spectroscopic analysis begins with isolation of a single ion, which is stored in a miniature electric trap. A laser is used to excite a broad resonance line from the ground state, and the emitted photons carry away portions of the ion's motional energy, leading to efficient cooling. The resulting reduction in thermal fluctuation improves the localization of the ion and eliminates frequency shifts produced by the Doppler effect. The fluorescence light that is scattered during laser cooling also permits the optical detection of the ion. In addition, the ion must possess a metastable level that couples to the ground state in a very narrow transition. This sharp line can then be used for precision measurements or can serve as a reference for an atomic clock. The excitation of the narrow transition is monitored in the fluorescence light emitted during cooling: If the ion is excited to the metastable level, it decouples from the cooling laser excitation and fluorescence ceases.

The requirement of having two transitions with very different linewidths that originate from a common ground state and can be excited with lasers at technically accessible wavelengths has limited these studies to only a handful of atomic ions.

Wineland *et al.* proposed a scheme (*6*) that relaxes the requirements by distributing the tasks to two different ions that are trapped together. The same group (*4*) now reports an experimental demonstration of the method. The narrow transition to be



Quantum logic spectroscopy. (**Left**) The "logic ion" (blue) is laser-cooled and the "spectroscopy ion" (yellow) is cooled sympathetically. (**Center**) A narrow transition of the spectroscopy ion is probed with a laser pulse. With additional laser pulses, the internal state of the spectroscopy ion is mapped to the logic ion via the common vibrational motion. (**Right**) The internal state of the logic ion can be read out via laser-induced fluorescence.

CREDIT: PRESTON HUEY/*SCIENCE*

studied is provided by a "spectroscopy ion" while laser cooling and state detection are performed with a different species, called the "logic ion" (see the figure). The two ions of like charge are coupled strongly via the Coulomb force, and at low enough temperature they form a quasi-molecule that is held together by the trap. Laser cooling of the logic ion will sympathetically cool the second ion, and the combined system will fall to the quantum ground state of the vibrational motion in the trap. The spectroscopy ion is now prepared for probing its narrow transition, but how is the success of an excitation attempt registered? A pulse from the spectroscopy laser maps the excitation of the spectroscopy ion to the vibrational mode.

It is here that the conditional aspect of quantum logic comes into play: An excitation of the vibration is produced only if the spectroscopy ion was internally excited. The procedure reliably transfers even the quantum mechanical coherence from one system to the other. In a second, similar step, the vibrational state is then mapped into two internal levels of the logic ion. The excitation of these levels can finally be read out via laser-induced fluorescence.

In the experiment reported here, Schmidt *et al*. have used $^{27}Al^+$ as the spectroscopy ion and $^{9}Be^+$ as the logic ion. The beryllium ion is convenient for laser cooling and has been used in a number of quantum logic experiments. Aluminum was chosen because it has a very narrow transition between two states with vanishing electronic angular momentum, which makes the transition especially immune to frequency shifts. This property is found in the ions of the third group of the periodic system, such as Tl^+ and In^+ (*7*), and makes them promising candidates for atomic clocks. The method demonstrated here is certainly not technically simple, but it is generalizable and makes a wide range of potentially interesting systems accessible to laser cooling and precision spectroscopy. Provided a suitable logic ion of similar mass can be found, the way is open to study any spectroscopy ion, including simple molecules as well as textbook examples such as He^+ with its hydrogen-like spectrum.

References

1. H. S. Margolis *et al*., *Science* **306**, 1355 (2004).
2. P. Gill *et al*., *Meas. Sci. Tech.* **14**, 1174 (2003).
3. S. A. Diddams, J. C. Bergquist, S. R. Jefferts, C. W. Oates, *Science* **306**, 1318 (2004).
4. P. O. Schmidt *et al*., *Science* **309**, 749 (2005).
5. H. G. Dehmelt, *IEEE Trans. Instrum. Meas.* **31**, 83 (1982).
6. D. J. Wineland *et al*., in *Proceedings of the 6th Symposium on Frequency Standards and Metrology*, P. Gill, Ed. (World Scientific, Singapore, 2002), pp. 361–368.
7. Th. Becker *et al*., *Phys. Rev. A* **63**, 051802(R) (2001).

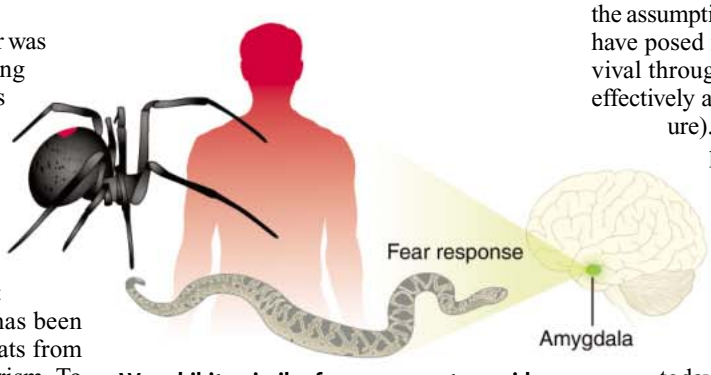
10.1126/science.1116127

PSYCHOLOGY

Conditioned Fear of a Face: A Prelude to Ethnic Enmity?

Arne Öhman

For all its absurdity, the Cold War was a political power game adhering to rational rules. However, it has been replaced by less predictable conflicts that give a larger role to emotion than to reason. They are manifested as vicious civil wars fueled by religious conflict and fought through terrorism and "ethnic cleansing." Thus, an abstract but overwhelming nuclear threat has been replaced by multiple concrete threats from "evil others" in the shape of terrorism. To help cope with this situation, we need a scientific understanding of the emotional dynamics of intergroup conflicts. An important priority is to understand how attitudes between ethnic groups are formed. Not surprisingly, it appears that fear plays an important role. On page 785 of this issue, Olsson *et al*. (*1*) show that fear conditioning, a simple and well-understood form of learning, results in lasting fear responses to outgroup but not to ingroup human faces. That is, white participants acquired more persistent conditioned fears in response to pictures of black faces than to pictures of white faces when the faces were paired with an aversive stimulus, whereas the opposite was true for black participants.



We exhibit a similar fear response to a spider, a snake, and a person of another race.

Fear has more insidious effects than to produce fright in response to a specific stimulus. Once we feel fear, we focus on escaping the situation rather than on in-depth evaluation of the real danger involved. Eventually, avoidance of not only the specific fear stimulus, but also of things associated with the dangerous situation, becomes based on anticipated rather than felt fear. In this way, avoidance precludes learning about a feared individual, making that person a blank slate for projections that serve to justify the fear. Hence, we are likely to demonize a feared person by thinking of the individual as dangerous, evil, and worthy of despise.

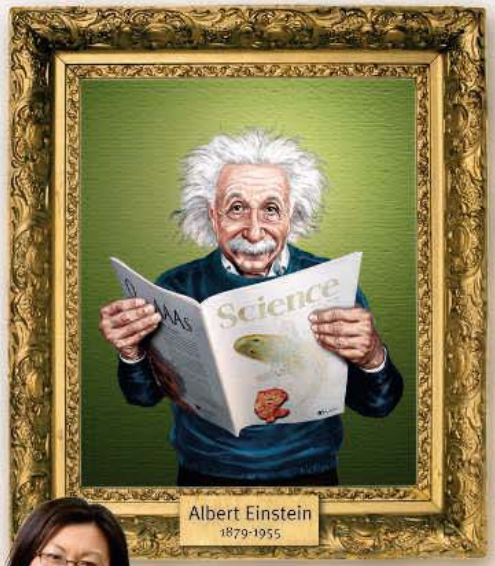
The research reported by Olsson *et al*. (*1*) was inspired by the notion of "preparedness" (*2*). It proposes that evolution has predisposed humans to more easily learn some things rather than others. With regard to fear, the assumption is that objects and events that have posed recurrent threats to human survival throughout evolution are rapidly and effectively associated with fear (see the figure). This mediates the evolutionary primed outcome, escape and avoidance (*3*). Accepting this theoretical premise, the results reported by Olsson *et al*. indicate that intergroup violence has been as common and damaging in evolution as it is among primates today (*4*). But claiming a biological basis for ethnic tension is contrary to the current emphasis on social learning. Indeed, the data reported by Olsson *et al*. (*1*) cannot distinguish between an evolutionary and a social learning origin of intergroup conflict. As the authors point out, their results may as easily be seen as effects, rather than causes, of a cultural climate that promotes interracial fears. Nevertheless, the pattern of findings is consistent with views stressing the social nature of human evolution in relatively small groups whose members are bound together by reciprocal altruism and a unique dialect or language. Thus, group cohesion may have been assisted by skepticism toward strangers and a readiness to develop fear of them.

This does not make racial conflict inevitable. The point of the preparedness

The author is in the Department of Clinical Neuroscience, Karolinska institutet, SE-17176 Stockholm, Sweden. He is currently a scholar at the Center for Advanced Studies of Behavioral Sciences, Stanford, CA 94305-8090, USA. E-mail: arne.ohman@cns.ki.se

Looking for a great science career?

Get the experts behind you.
Visit www.ScienceCareers.org



Your career is too important to leave to chance. So to find the right job or get career advice, turn to the experts. At ScienceCareers.org we know science. And we are committed to helping take your career forward. Our knowledge is firmly founded on the expertise of *Science*, the premier scientific journal, and the long experience of AAAS in advancing science around the world. Put yourself in the picture with the experts in science. Visit www.ScienceCareers.org.

ScienceCareers.org
We know science AAAS

concept (*2*) may be obvious even though it is not part of current common sense: Biology channels us in some directions rather than others, but learning is required to produce a definite outcome. And what is learned can be unlearned. Indeed, the fear conditioning effect observed by Olsson *et al*. was attenuated by the experience of interracial dating (*1*).

Fear conditioning appears to result automatically from pairing a neutral stimulus with an aversive one. In humans, it does not require conscious mediation (*3*). For example, human fear conditioning has been demonstrated in response to faces that were blocked from awareness by backward masking (*5*–*7*). This is consistent with contemporary analyses of attitude formation that stress the distinction between implicit (nonconscious) and explicit (conscious) attitudes (*8*). Implicit racial prejudice has been demonstrated in participants who do not endorse explicitly prejudiced statements about another group but who nevertheless show a negative bias in an implicit evaluation task. For example, without realizing it, white participants more readily associate negative features with black than with white stimulus persons (*9*, *10*).

Brain imaging studies show that both fear conditioning (*6, 7*) and implicit negative attitudes to outgroup faces (*9*) correlate with activity in the amygdala even when the faces are masked from conscious recognition. Thus, negative emotional responses to members of a different race are independent of conscious mentation, which may make such responses relatively immune to rational persuasion. However, there is a consistent finding that indices of interracial contact show inverse relationships to both fear conditioning (*1*) and amygdala activation (*9*) in response to outgroup faces.

The findings of Olsson *et al*. show that negative attitudes to other races partly derive from consciously inaccessible emotional processes that may be difficult to affect by rational deliberations and decisions. However, these processes can be counteracted by interracial exposure and contact. Furthermore, understanding the ease of associating negative emotions with features defining an outgroup may help combat the development of emotionally charged stereotypes of outgroup members.

References

1. A. Olsson, J. P. Ebert, M. R. Banaji, E. A. Phelps, *Science* **309**, 785 (2005).
2. M. E. P. Seligman, *Psychol. Rev.* **77**, 406 (1970).
3. A. Öhman, S. Mineka, *Psychol. Rev.* **108**, 483 (2001).
4. R. Wrangham, D. Peterson, *Demonic Males. Apes and the Origins of Human Violence* (Bloomsbury, London, 1996).
5. F. Esteves, C. Parra, U. Dimberg, A. Öhman, *Psychophysiology* **31**, 375 (1994).
6. H. D. Critchley, C. J. Mathias, R. J. Dolan, *Neuron* **33**, 653 (2002).
7. J. S. Morris, C. Büchel, R. J. Dolan, *Neuroimage* **13**, 1052 (2001).
8. A. G. Greenwald, M. R. Banaji, *Psychol. Rev.* **102**, 4 (1995).
9. W. A. Cunningham *et al.*, *Psychol. Sci.* **15**, 806 (2004).
10. E. A. Phelps *et al.*, *J. Cogn. Neurosci.* **12**, 729 (2000).

10.1126/science.1116710

CHEMISTRY

Oxygen Vacancies and Catalysis on Ceria Surfaces

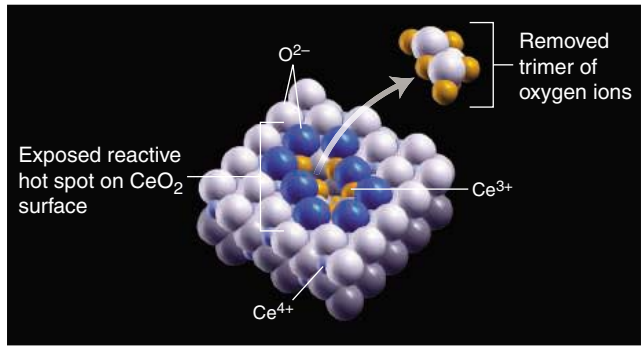
Charles T. Campbell and Charles H. F. Peden

Chemistry that occurs at the surfaces of metal oxides is critical in a variety of industrial applications including catalysis, optical display technology, solar energy devices, and corrosion prevention. Defects such as oxygen vacancies and step edges are the most reactive sites on the surfaces of metal oxides. Understanding metal-oxide reactivity thus requires an understanding of the nature of surface oxygen vacancies, and their number, distribution, and diffusion across the surface. On page 752 of this issue, Esch and co-workers report an exciting study that clearly elucidates the structure, distribution, and formation of oxygen vacancies on a cerium oxide surface (*1*).

CeO_2 is one of the most interesting oxides industrially because oxygen vacancy defects can be rapidly formed and eliminated, giving it a high "oxygen storage capacity." It is this capacity that makes modern automotive exhaust treatment catalysts containing CeO_2 much more effective than their predecessors without CeO_2. Relative to other oxide supports, ceria also enhances the performance of transition metal catalysts in a variety of other reactions including water-gas shift, steam reforming of oxygenates, and PROX (preferential oxidation of CO) (*2*–*6*), all of which hold promise for enabling a hydrogen economy (*2*). Surprisingly, using some less reducible oxides, such as zirconia (ZrO_2), as additives enhances the "oxygen storage" property of CeO_2.

To gain new insight into CeO_2 surfaces, Esch and co-workers elegantly combine beautiful, atomic-resolution imaging using scanning tunneling microscopy (STM) on a ceria surface with state-of-the-art quantum mechanical calculations using density functional theory (DFT). They show that surface oxygen vacancies on CeO_2(111) are immobile at room temperature, but linear clusters of these vacancies form at higher temperatures. These vacancy clusters expose exclusively Ce^{3+} ions to gas-phase reactants. Thus, exposed Ce^{3+} ions are grouped into large ensembles, whereas the sites immediately adjacent to these vacancy clusters remain as pure Ce^{4+} ions (see the figure). The authors further show that one subsurface oxygen vacancy is required to nucleate each vacancy cluster. Guided by this knowledge, they performed DFT calculations that suggest an exciting new explanation for the role of Zr promoters in ceria-based catalysts: to enable growth of the linear vacancy chains without the need for a subsurface vacancy, which is energetically more costly than a surface vacancy. Namai *et al*. (*7, 8*) also recently reported such linear vacancy clusters on CeO_2(111), for which Esch *et al*. now provide much needed atomic-level structural detail.

Surface oxygen vacancies are proposed to participate in many chemical reactions catalyzed by metal oxides. For example, when an adsorbate is oxidized at the surface, the oxidant is often a surface lattice oxygen



Reactive defect in a ceria surface. Coordinated Ce^{4+} ions in a dimeric surface vacancy cluster are reduced to reactive Ce^{3+} when the trimer of oxygen ions (surface dimer plus a subsurface oxygen) is removed.

The authors are in the Chemistry Department, University of Washington, Seattle, WA 98195, USA, and the Institute for Interfacial Catalysis, Pacific Northwest National Laboratory, Richland, WA 99352, USA. E-mail: campbell@chem.washington.edu

atom, thus creating a surface oxygen vacancy (the Mars/van Krevelen mechanism). Vacancies also bind adsorbates more strongly than normal oxide sites and assist in their dissociation (*9, 10*). Oxygen vacancies are also involved in photocatalysis on oxides (*9–12*), and their charged nature may control band-bending and thus electron-hole pair separation. These groups of exposed Ce^{3+} ions on CeO_2 are a potentially potent surface site for catalysis and photocatalysis, because adsorbed gases or catalytic reaction intermediates could interact simultaneously with several Ce^{3+} ions. Because oxygen vacancies stabilize transition metal nanoparticles supported on oxide surfaces (*13, 14*), such linear vacancy clusters also may be important in stabilizing CeO_2-supported transition metal catalysts against sintering, and might even direct the metal nanoparticles into specific shapes (that is, nanowires) that may have advantages in catalysis.

STM images of well-ordered TiO_2(110) surfaces have uncovered a surprising amount of detail about oxygen vacancies and adsorption on that important oxide (*15–19*). This has led to a blossoming in our understanding of surface chemistry on TiO_2 over the past decade. The Esch *et al.* findings (*1*) and those of Fukui and Iwasawa's group (*7*, *8*, *20*) promise a similar unfolding in our atomic-level understanding of CeO_2 surfaces. Interestingly, vacancies also tend to cluster into linear chains on TiO_2(110) (*16*), which suggests this may be a general phenomenon on such easily reducible oxides (for which the energetic cost of oxygen loss is low). Indeed, vacancy clustering would be consistent with commonly observed bulk-phase segregations of oxides into domains with lower and higher extents of reduction on larger length scales.

The room-temperature mobility of single oxygen vacancies on CeO_2 remains controversial. They were reported to be highly mobile on CeO_2(111) by Namai *et al.* (*7, 8*), but Esch *et al.* (*1*) now find them completely immobile. They also have been reported to be very mobile on TiO_2(110) (*16*), but catalytic properties suggest higher vacancy mobility in CeO_2 than in TiO_2. Might defects seen in STM images have been misidentified as simple oxygen vacancies in some of these studies?

Recently Reuter *et al.* (*21*) and Honkala *et al.* (*22*) demonstrated the contribution of DFT to elucidating the energetics, thermodynamics, and kinetic parameters of surface reactions. Now, Esch *et al.* highlight its complementary role in enabling structurally detailed interpretations of STM images and other experimental measurements of surface structure. Its further application may clarify the ongoing vacancy mobility controversy.

References

1. F. Esch *et al.*, Science **309**, 752 (2005) .
2. R. Farrauto *et al.*, *Annu. Rev. Mater. Res.* **33**, 1 (2003).
3. C. Wheeler *et al.*, *J. Catal.* **223**, 191 (2004).
4. Q. Fu, H. Saltsburg, M. Flytzani-Stephanopoulos, *Science* **301**, 935 (2003).
5. T. Bunluesin, R. J. Gorte, G. W. Graham, *Appl. Catal. B* **15**, 107 (1998).
6. G. A. Deluga, J. R. Salge, L. D. Schmidt, X. E. Verykios, *Science* **303**, 993 (2004).
7. Y. Namai, K. I. Fukui, Y. Iwasawa, *Catal. Today* **85**, 79 (2003).
8. Y. Namai, K. Fukui, Y. Iwasawa, *J. Phys. Chem. B* **107**, 11666 (2003).
9. G. Lu, A. Linsebigler, J. T. Yates, *J. Phys. Chem.* **99**, 7626 (1995).
10. M. A. Henderson *et al.*, *J. Phys. Chem. B* **103**, 5328 (1999).
11. H. Uetsuka, H. Onishi, M. A. Henderson, J. M. White, *J. Phys. Chem. B* **108**, 10621 (2004).
12. M. A. Henderson, J. M. White, H. Uetsuka, H. J. Onishi, *J. Am. Chem. Soc.* **125**, 14974 (2003).
13. L. Giordano, J. Goniakowski, G. Pacchioni, *Phys. Rev. B* **64**, 075417 (2001).
14. Y. D. Kim, J. Stultz, T. Wei, D. W. Goodman, *J. Phys. Chem. B* **106**, 6827 (2002).
15. E. Wahlstrom *et al.*, *Science* **303**, 511 (2004).
16. R. Schaub *et al.*, *Science* **299**, 377 (2003).
17. U. Diebold, *Surf. Sci.* **578**, 1 (2005).
18. S. Lee, C. Y. Fan, T. P. Wu, S. L. Anderson, *Surf. Sci.* **578**, 5 (2005).
19. M. Sander, T. Engel, *Surf. Sci.* **302**, L263 (1994).
20. S. Gritschneder, Y. Namai, Y. Iwasawa, M. Reichling, *Nanotechnology* **16**, S41 (2005).
21. K. Reuter, D. Frenkel, M. Scheffler, *Phys. Rev. Lett.* **93**, 116105 (2004).
22. K. Honkala *et al.*, *Science* **307**, 555 (2005).

23. This work was supported by the U.S. Department of Energy, Office of Basic Energy Sciences, Chemical Sciences Division. We thank the authors of (*1*) for kindly generating the figure using XCRYSDEN (www.xcrysden.org).

10.1126/science.1113955

ASTRONOMY

Very Energetic Gamma Rays from Microblazars

Wei Cui

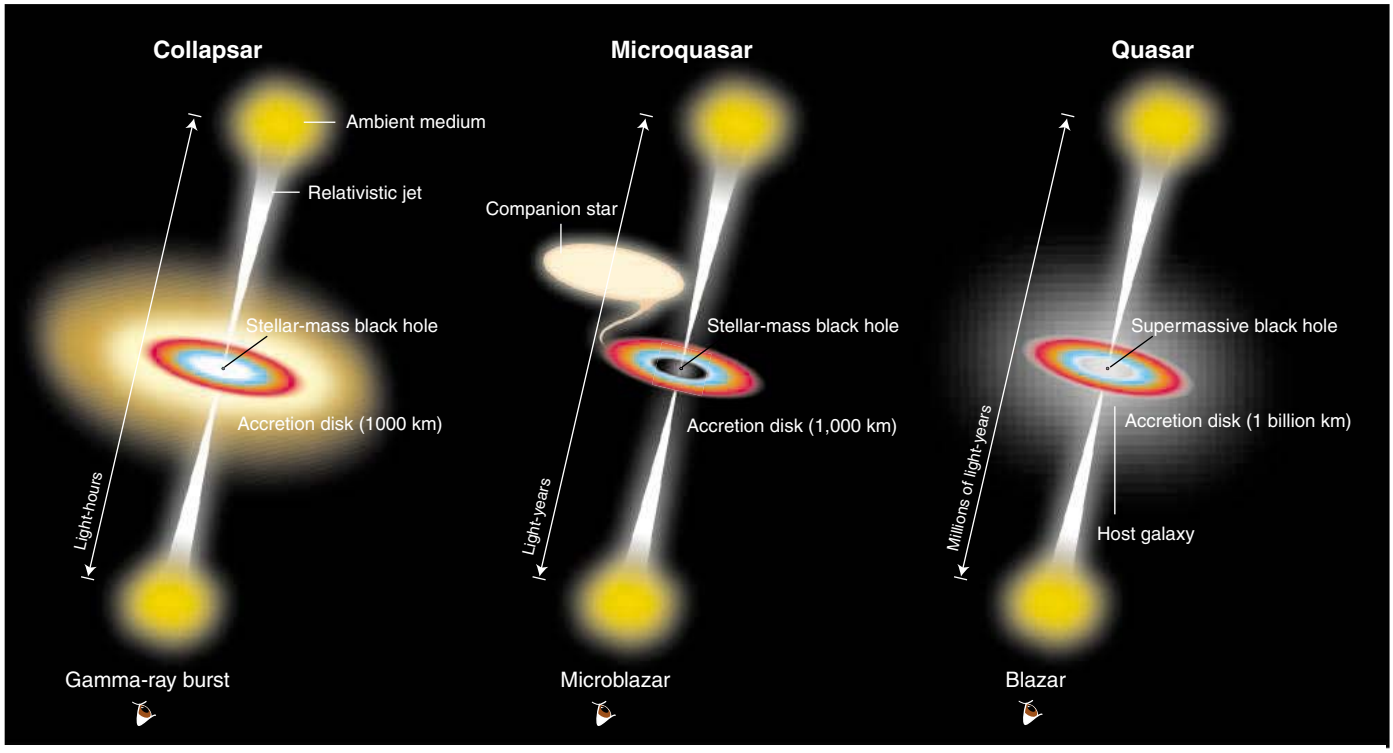
Active galactic nuclei (AGN) are some of the most powerful objects in the universe, and they are almost certainly powered by very massive black holes at the center of galaxies. As clouds, stars, or other material fall into the black hole, some of their gravitational energy is converted into radiation that we detect on Earth. The accretion of matter by the black hole is sometimes accompanied by well-collimated and powerful outflows of material, also known as jets. Blazars are an important subset of AGN whose jets are closely aligned with our line of sight (see the figure). On page 746 of this issue, Aharonian *et al.* report the possible detection of a long-sought downsized version of these objects called a "microblazar" (*1*).

The author is in the Department of Physics, Purdue University, West Lafayette, IN 47907, USA. E-mail: cui@physics.purdue.edu

Such microblazars could hypothetically exist, given that there is growing observational evidence for the presence of micro-AGN (*2*), powered by black holes less than one-millionth the mass of those in blazars. These micro-AGN belong to a more general class of sources known as x-ray binaries, which are some of the brightest x-ray sources in the sky. An x-ray binary consists of a stellar-mass black hole or a neutron star and a normal star bound together by their mutual gravitational attraction. If a black hole system also produces jets, it is referred to as a microquasar, and many have been discovered over the past decade. A microquasar whose jet points at us would be a microblazar (see the figure). Although circumstantial evidence exists, the presence of microblazars has not yet been firmly established observationally.

One way to find microblazars is perhaps to look for very energetic gamma rays from known (or candidate) microquasars. Some positive detections were claimed but were nearly always disputed by independent observations with a different (sometimes more sensitive) instrument or dismissed on other grounds. It is fair to say that there has not be any credible evidence for the detection of TeV gamma rays from any microquasar until now. Aharonian *et al.* (*1*) present a careful analysis of the fields in their survey of the Milky Way with the High Energy Stereoscopic System (HESS) array that covered the position of a microblazar candidate, LS 5039. The image they show indicates a clear detection of the source at TeV energies. The question remains whether LS 5039 is truly a microblazar. The authors are cautious in this regard.

LS 5039 is considered a microblazar candidate because it has been identified as a counterpart to one of the still-mysterious sources detected at GeV energies by the Energetic Gamma Ray Experiment Telescope (EGRET) instrument aboard the Compton Gamma Ray Observatory. It is worth noting that most of the identified EGRET sources are in fact blazars. LS 5039 was subsequently observed and detected at x-ray and radio wavelengths. An important



Powerful sources. Gamma-ray bursts, microblazars, and blazars are all powered by a black hole via mass accretion, and all produce jets that are aligned with our line of sight. [Adapted from from (*8*)]

recent breakthrough came from direct imaging of the jets in the source at radio wavelengths (*3*), lending support to its microblazar candidacy. However, unlike typical blazars, the jets in LS 5039 appear to be only very mildly relativistic; the effects of relativistic beaming, which play an important role in blazars, should therefore not be relevant here. Nevertheless, the detection of radio jets in LS 5039 has generated a lot of excitement and led to detailed studies of physical mechanisms to produce very energetic gamma rays in microblazars (*4*, *5*).

There are two schools of thought regarding the origin of gamma-ray emission from blazars and microblazars. If the jets are composed purely of electron-positron pair plasma, gamma rays may be emitted by relativistic electrons or positrons as they scatter low-energy photons up to very high gamma-ray energies. If the jets are composed of normal plasma (mostly electrons and protons), on the other hand, gamma rays may be produced in the interaction between ultrarelativistic protons and low-energy photons or protons. The calculations show that either mechanism could account for the GeV gamma rays from LS 5039, as detected by EGRET, and also predict the level of TeV gamma-ray emission that might become detectable with the next-generation TeV observatories such as HESS (*4*–*6*).

Despite all of the recent progress, our understanding of jets in AGN or microAGN is embarrassingly inadequate. For example, we think that jets are somehow connected to accretion flows, but we are not sure how exactly they are formed or collimated; we do not understand how particles in the jets are accelerated to energies required to produced the observed gamma-ray emission; and, except for one microquasar (SS 433), we know little about the composition of the jets. Observations at TeV energies have the potential to cast considerable light on the last two issues. Knowledge may also be gained by studying and comparing blazars and microblazars, because similar physical processes are thought to operate in both types of systems. From a practical standpoint, each type offers advantages and disadvantages observationally, so it is more effective to observe and study them as a group. There is strong evidence that yet another class of sources, gamma-ray bursts (GRBs), may have a similar physical configuration but on even smaller scales (see the figure). Dubbed "the most powerful explosions since the Big Bang," GRBs are thought to be powered also by stellar-mass black holes but in a highly explosive manner. No GRBs have been detected at TeV energies yet, but they remain promising targets for more sensitive facilities like HESS.

The field of TeV gamma-ray astronomy has gradually grown out of its infancy and emerged as a viable branch of modern astronomy [see (*7*) for a detailed account of the development]. One of the key goals now is to understand particle acceleration and radiation mechanisms in the jets of black hole systems. Microblazars, blazars, and GRBs together provide an excellent laboratory for carrying out such studies over a wide range of physical scales, from light-hours to thousands of light-years. Besides HESS, two other new TeV observatories, Major Atmospheric Gamma Imaging Cherenkov (MAGIC) and Collaboration of Australia and Nippon for a Gamma Ray Observatory in the Outback (CANGAROO) III, are also operational, and a third, Very Energetic Radiation Imaging Telescope Array System (VERITAS), is expected to come online next year. Together, these state-of-the-art observatories will cover both northern and southern skies with unprecedented sensitivities.

References

1. F. Aharonian *et al.*, *Science* **309**, 746 (2005); published online 7 July 2005 (10.1126/science.1113764).
2. I. F. Mirabel, L. F. Rodriguez, *Annu. Rev. Astron. Astrophys.* **37**, 409 (1999).
3. J. M. Paredes, J. Marti, M. Ribo, M. Massi, *Science* **288**, 2340 (2000).
4. M. M. Kaufman Bernadó, G. E. Romero, I. F. Mirabel, *Astron. Astrophys.* **385**, L10 (2002).
5. G. E. Romero, D. F. Torres, M. M. Kaufman Bernadó, I. F. Mirabel, *Astron. Astrophys.* **410**, L1 (2003).
6. V. Bosch-Ramon, J. M. Paredes, *Astron. Astrophys.* **417**, 1075 (2004).
7. T. C. Weekes, *Very High Energy Gamma-Ray Astronomy* (Institute of Physics, Bristol, UK, 2003).
8. I. F. Mirabel, L. F. Rodriguez, *Sky & Telescope* (May 2002), p. 32.

10.1126/science.1116381

edited by Edward W. Lempinen

INTERNATIONAL

High-Level AAAS Visit to China Yields Likely New Collaboration

Top AAAS executives returned from a 6-day visit to China deeply impressed with the nation's commitment to science and technology, and optimistic that their meetings with high-level Chinese S&T leaders would yield a range of new cooperative ventures.

AAAS officials said talks would continue in the weeks ahead and that they expected agreements to work together on science education, public engagement in science, and sustainability, among other issues.

"There is a tremendous commitment from all quarters in China to significantly advancing the pace of science and its application to foster innovation and improve the quality of life," said Alan I. Leshner, the CEO of AAAS and executive publisher of *Science*. "There are excellent opportunities for collaboration and mutual education among our scientific communities. Our discussions with the Chinese scientific leadership yielded a clear commitment to that collaboration and cooperation."

Lu Yongxiang, president of the Chinese Academy of Sciences (CAS), was similarly hopeful after the meetings that the AAAS visit would foster dialogue and collaboration between the science and engineering communities in each nation. Scientists in both countries share common interests, Lu said, including the shortage of energy resources, exploration of the deep oceans and outer space, the roots of human consciousness, nanotechnology, and the need to develop a science of sustainability.

"The globalization of the economy and the development of an information and network society provide us an unprecedented environment and conditions for cooperation," Lu said in an e-mail interview. "The United States is the most developed industrial society in the world, while China is the fastest developing country with the largest population. It is complementary to each other's advantage and benefits for both China and the U.S. to cooperate. Scientists from both sides shoulder more responsibility for cooperation between the two countries, as well as for mankind. Both CAS and AAAS should and could play an important role to facilitate such cooperation."

Lu also is vice-chairman of the Standing Committee of the National People's Congress, responsible especially for Science, Education, Healthcare, and Culture.

The trip marked Leshner's first official visit to China since becoming CEO in December 2001. The AAAS delegation also included Chief International Officer Shere Abbott and Education and Human Resources Director Shirley Malcom.

During the 6-day visit (18 to 23 June), AAAS officials met with some of China's most influential science, education, and engineering leaders. At the China Association for Science and Technology (CAST), AAAS's counterpart and host of the visit, the delegation met with Secretary-General Cheng Donghong; Vice President Deng Nan, the former vice minister of Science and Technology; and Vice President Wei Yu, former vice minister of Education.

Lu Yongxiang, president of the Chinese Academy of Sciences, with Alan I. Leshner, the CEO of AAAS and executive publisher of *Science*.

The delegation also met with Xu Guanhua, the Chinese minister of Science and Technology; Zhao Qinping, vice minister of Education; Chen Yiyu, president of the National Natural Science Foundation of China; Xu Kuangdi, president of the Chinese Academy of Engineering; and Huang Ping, director of international affairs at the Chinese Academy of Social Sciences.

AAAS

New AAAS Dues Rates Approved for 2006

The AAAS Board of Directors has approved a dues increase for 2006. The Board authorizes increases to cover two kinds of expenses: unavoidable costs associated with running AAAS and publishing *Science*, and new expenses that add value to membership. Postage and paper increases and improving online resources are examples of the kind of expenses the Board anticipated in setting the 2006 rates.

The new rates are effective for terms beginning after 31 December 2005. As listed below, they do not include postage or taxes for international members, which is additional.

Regular professional members	$139
Postdocs and K-12 teachers	$99
Emeritus members who receive print *Science*	$110
Students	$75
Patrons	$300
Supporting and Emeritus members who do not receive *Science*	$56*

The Board also set the institutional subscription rate for print *Science* at $360 for high school and public libraries and $650 for all other institutions. For further information, including subscription rates for *Science* Online, librarians should contact AAAS or their subscription agents, or go to www.sciencemag.org/subscriptions/inst-sol-access.dtl on the Web.

All members will be advised of the new dues rates on their renewal notices for 2006. Member dues and voluntary contributions form the critical financial base for a wide range of AAAS activities. For more information, contact the AAAS Membership Office at 202-326-6417, or www.aaas.org/membership/.

* Supporting member dues rate is set by the membership department.

What emerged from the talks was a consensus that the U.S. and China, despite their differences, share significant common challenges. Science and technology will be central to continued economic growth and the development of a more sustainable economy. To help achieve that, science education and public science literacy will be essential.

AAAS officials are conferring with CAST on a memorandum of understanding encompassing three strategic areas—science education, communicating science to the public, and sustainability. The agreement could allow AAAS to work through CAST to build collaborative relations throughout the Chinese S&T culture. AAAS is already sharing Project 2061 curriculum materials with China, and that may expand.

Chinese officials also pressed their concerns that the U.S. visa system imposes unnecessary restrictions on Chinese and other scholars who want to visit or study in the United States. AAAS and other science and education groups having been working with the U.S. Departments of State and Homeland Security for over a year on easing visa rules.

The United States "should look far into the future and more actively support free cooperation and exchanges between the scientists of the two countries in various scientific disciplines," Lu said. "In addition, both sides should pay particular attention to the strengthening of cooperation and exchanges between young scientists, thus making the Sino-U.S. cooperation in S&T develop and grow on a solid and sustainable basis."

SCIENCE AND POLICY

AAAS: Commerce Proposal Would Hurt U.S. Research

AAAS has joined with major education organizations in expressing concern about a Commerce Department proposal to tighten restrictions on access by foreign nationals to sensitive technologies at U.S. research universities.

If approved, the proposal by the department's Bureau of Industry and Security would further discourage top-notch foreign students and scholars from coming to U.S. universities, Albert H. Teich, AAAS's director of Science and Policy Programs, wrote in a letter. Further, he said, the proposed new rules would impose a costly new bureaucracy on the universities, which would be required to enforce the regulations.

"While AAAS understands Commerce's interest in protecting the commercial transfer of technologies to certain nations," Teich wrote, "the Association believes that the [proposed rules] will further restrict the conduct of fundamental research and diminish our national security rather than increase it."

The Bureau of Industry and Security is considering proposed changes to its "deemed export" rules intended to restrict the export of knowledge about sensitive technology to nationals of certain countries. The rules would require universities to assess their research equipment to determine whether federal licenses for foreign nationals are required before those researchers could work on sensitive equipment.

The proposal was based on a March 2004 study by the Inspectors General of the Departments of Commerce and Defense which concluded that foreign nationals' access to sensitive technology poses a potential threat to U.S. security.

The Association of American Universities and the Council on Governmental Relations, an association of U.S. research universities, also have strongly questioned the proposed changes. In the AAAS letter, Teich said that existing regulations are sufficient to preserve national security.

"Foreign nationals who apply for student visas already must submit to an extensive examination by State Department consular offices and to Visas Mantis screening," Teich wrote. "Requiring an additional layer of scrutiny by institutions is overly burdensome and unnecessary."

"The impact of the proposed revisions on scientific research and our nation's economic competitiveness would be substantial," Teich concluded, "while expected improvements to national security have not been persuasively presented by the Department of Commerce. To the extent that the proposed changes lead to delays or unnecessary denials of licenses for foreign nationals seeking to work on fundamental research in the U.S., they have the potential to set back research, alienate foreign scholars and students, and exacerbate the declining enrollment of foreign nationals in U.S. science and engineering graduate school."

The full letter can be seen at www.aaas.org/news/releases/2005/0706de.pdf.

ELECTIONS

AAAS Annual Election: Preliminary Announcement

The 2005 AAAS election of general and section officers will be held in September. All members will receive a ballot for election of the president-elect, members of the Board of Directors, and members of the Committee on Nominations. Members registered in one to three sections will receive ballots for election of the chair-elect, member-at-large of the Section Committee, and members of the Electorate Nominating Committee for each section.

Members enrolled in the following sections will also elect Council delegates: Agriculture, Food, and Renewable Resources; Engineering; History and Philosophy of Science; Industrial Science and Technology; Medical Sciences; Psychology; and Social, Economic, and Political Sciences.

Candidates for all offices are listed below. Additional names may be placed in nomination for any office by petition submitted to the Chief Executive Officer no later than 13 September. Petitions nominating candidates for president-elect, members of the Board, or members of the Committee on Nominations must bear the signatures of at least 100 members of the Association. Petitions nominating candidates for any section office must bear the signatures of at least 50 members of the section. A petition to place an additional name in nomination for any office must be accompanied by the nominee's curriculum vitae and statement of acceptance of nomination.

Biographical information for the following candidates will be enclosed with the ballots mailed to members in September.

Slate of Candidates

GENERAL ELECTION

President-Elect: David Baltimore, California Institute of Technology; Karen A. Holbrook, Ohio State Univ., Columbus.

Board of Directors: Denice D. Denton, Univ. of California, Santa Cruz; Alice P. Gast, Massachusetts Institute of Technology; Daniel J. Kevles, Yale Univ.; Thomas D. Pollard, Yale Univ.

Committee on Nominations: Elizabeth Blackburn, Univ. of California, San Francisco; John I. Brauman, Stanford Univ.; Peter G. Brewer, Monterey Bay Aquarium Research Institute, Moss Landing, CA; R. James Cook, Washington State Univ.; Claire M. Fraser, The Institute for Genomic Research, Rockville, MD; Pauline O. Lawrence, Univ. of Florida; John H. Seinfeld, California Institute of Technology; Richard A. Tapia, Rice Univ.

SECTION ELECTIONS

Agriculture, Food, and Renewable Resources
Chair-Elect: Terry D. Etherton, Pennsylvania State Univ.; James L. Van Etten, Univ. of Nebraska, Lincoln.
Member-at-Large of the Section Committee: Noelle E. Cockett, Utah State Univ.; Eugene Nester, Univ. of Washington.
Electorate Nominating Committee: Werner G. Bergen, Auburn Univ.; Thomas J. Guilfoyle, Univ. of Missouri, Columbia; Paul A. Lachance, Rutgers Univ.; George E. Seidel Jr., Colorado State Univ.
Council Delegate: Harris A. Lewin, Univ. of Illinois, Urbana-Champaign; Jeffrey C. Silvertooth, Univ. of Arizona.

Anthropology
Chair-Elect: A. Theodore Steegmann Jr., State Univ. of New York, Buffalo; William A. Stini, Univ. of Arizona.
Member-at-Large of the Section Committee: Cynthia M. Beall, Case Western Reserve Univ.; Susan Cachel, Rutgers Univ.
Electorate Nominating Committee: John Kappelman, Univ. of Texas, Austin; Timothy A. Kohler, Washington State Univ.; Jeffrey H. Schwartz, Univ. of Pittsburgh; Lynnette Leidy Sievert, Univ. of Massachusetts, Amherst.

Astronomy
Chair-Elect: Alyssa A. Goodman, Harvard Univ.; Margaret Galland Kivelson, Univ. of California, Los Angeles.
Member-at-Large of the Section Committee: Julie Lutz, Univ. of Washington; Hugh M. Van Horn, Alexandria, VA.
Electorate Nominating Committee: Karen S. Bjorkman, Univ. of Toledo; Steven Kilston, Ball Aerospace and Technologies Corp., Boulder; Joel E. Tohline, Louisiana State Univ.; Alma C. Zook, Pomona College, Claremont, CA.

Atmospheric and Hydrospheric Sciences
Chair-Elect: Thomas P. Ackerman, Pacific Northwest National Lab.; Thomas E. Graedel, Yale Univ.
Member-at-Large of the Section Committee: Alan Robock, Rutgers Univ.; Peter J. Webster, Georgia Institute of Technology.
Electorate Nominating Committee: Kristie A. Boering, Univ. of California, Berkeley; David D. Houghton, Univ. of Wisconsin, Madison; Mary Jane Perry, Univ. of Maine, Walpole; David A. Randall, Colorado State Univ.

Biological Sciences
Chair-Elect: Richard I. Morimoto, Northwestern Univ.; Virginia Walbot, Stanford Univ.
Member-at-Large of the Section Committee: Marian Carlson, Columbia Univ.; Malcolm Potts, Virginia Polytechnic Institute and State Univ.
Electorate Nominating Committee: Lee-Ann H. Allen, Univ. of Iowa; Susan L. Hamilton, Baylor College of Medicine; Daphne Preuss, Univ. of Chicago; Barbara T. Wakimoto, Univ. of Washington.

Chemistry
Chair-Elect: Jon Clardy, Harvard Medical School; John C. Hemminger, Univ. of California, Irvine.
Member-at-Large of the Section Committee: Robin L. Garrell, Univ. of California, Los Angeles; Patricia A. Thiel, Iowa State Univ.
Electorate Nominating Committee: Paul L. Houston, Cornell Univ.; Marisa C. Kozlowski, Univ. of Pennsylvania; David A. Tirrell, California Institute of Technology; Gregory A. Voth, Univ. of Utah.

Dentistry and Oral Health Sciences
Chair-Elect: Ann Progulske-Fox, Univ. of Florida; Malcolm L. Snead, Univ. of Southern California.
Member-at-Large of the Section Committee: Beverly A. Dale-Crunk, Univ. of Washington; Richard J. Lamont, Univ. of Florida.
Electorate Nominating Committee: Susan Kinder Haake, Univ. of California, Los Angeles; Ichiro Nishimura, Univ. of California, Los Angeles; Frank A. Scannapieco, State Univ. of New York, Buffalo; Philip Stashenko, Forsyth Institute, Boston.

Education
Chair-Elect: Daryl E. Chubin, AAAS; James H. Stith, American Institute of Physics, College Park, MD.
Member-at-Large of the Section Committee: Arthur Eisenkraft, Univ. of Massachusetts, Boston; Penny J. Gilmer, Florida State Univ.
Electorate Nominating Committee: Sandra K. Abell, Univ. of Missouri, Columbia; Bonnie J. Brunkhorst, California State Univ., San Bernardino; David Lavalle, State Univ. of New York, New Paltz; William F. Polik, Hope College, Holland, MI.

Engineering
Chair-Elect: Richard J. Goldstein, Univ. of Minnesota, Minneapolis; Gail H. Marcus, Organization of Economic Cooperation and Development, Paris.
Member-at-Large of the Section Committee: Herbert H. Richardson, Texas A&M Univ.; Jerome S. Schultz, Univ. of California, Riverside.
Electorate Nominating Committee: Sangeeta N. Bhatia, Massachusetts Institute of Technology; Frank L. Huband, American Society for Engineering Education, Washington, DC; Herbert Voigt, Boston Univ.; Gordana Vunjak-Novakovic, Columbia Univ.
Council Delegates: James J. Duderstadt, Univ. of Michigan, Ann Arbor; Janie Fouke, Michigan State Univ.; Winfred M. Phillips, Univ. of Florida; Yoram Rudy, Washington Univ.

General Interest in Science and Engineering
Chair-Elect: Lynn E. Elfner, Ohio Academy of Science, Columbus; Gary King, Harvard Univ.
Member-at-Large of the Section Committee: Suzanne Gage Brainard, Univ. of Washington; Linda Trocki, Bechtel National, Inc., San Francisco.
Electorate Nominating Committee: Jack O. Burns, Univ. of Colorado, Boulder; Robert D. Crangle, Rose & Crangle, Ltd., Lincoln, KS; John Katzenberger, Aspen Global Change Institute, Aspen, CO; Kellogg J. Schwab, Johns Hopkins Univ.

Geology and Geography
Chair-Elect: James C. Knox, Univ. of Wisconsin, Madison; John D. Rummel, NASA Headquarters, Washington, DC.
Member-at-Large of the Section Committee: Greg Ravizza, Univ. of Hawaii, Honolulu; Carol Stoker, NASA Ames Research Center, Moffett Field, CA.
Electorate Nominating Committee: Paul A. Baker, Duke Univ.; Samantha B. Joye, Univ. of Georgia; George P. Malanson, Univ. of Iowa; Debra A. Willard, U.S. Geological Survey, Reston, VA.

History and Philosophy of Science
Chair-Elect: James R. Fleming, Colby College, Waterville, ME; Elliott Sober, Univ. of Wisconsin, Madison.
Member-at-Large of the Section Committee: Douglas Allchin, Univ. of Minnesota, Minneapolis; Robert N. Brandon, Duke Univ.
Electorate Nominating Committee: Daniel P. Jones, Kensington, MD; Paul R. Josephson, Colby College, Waterville, ME; Corinna Treitel, Washington Univ.; Virginia Trimble, Univ. of California, Irvine.
Council Delegate: Sara Joan Miles, Eastern Univ., St. Davids, PA; Nicolas Rasmussen, Univ. of New South Wales, Sydney.

Industrial Science and Technology
Chair-Elect: Anice I. Anderson, Rose-Hulman Institute of Technology, Terre Haute, IN; Charles L. Liotta, Georgia Institute of Technology.
Member-at-Large of the Section Committee: David Cheney, SRI International, Washington, DC; Max G. Lagally, Univ. of Wisconsin, Madison.

Electorate Nominating Committee: Fran Adar, Jobin Yvon, Inc., Edison, NJ; Kevin W. Bieg, Sandia National Labs.; Thomas W. Eagar, Massachusetts Institute of Technology; Kenneth A. Jackson, Univ. of Arizona.
Council Delegate: Attilio Bisio, West Park Associates, LLC, Westfield, NJ; Steven W. Popper, RAND Corp., Santa Monica.

Information, Computing, and Communication
Chair-Elect: Tora K. Bikson, RAND Corp., Santa Monica; Bryant W. York, Portland State Univ.
Member-at-Large of the Section Committee: Joel Moses, Massachusetts Institute of Technology; Bruce R. Schatz, Univ. of Illinois, Urbana-Champaign.
Electorate Nominating Committee: L. Jean Camp, Indiana Univ., Bloomington; Barbara J. Grosz, Harvard Univ.; Sartaj Sahni, Univ. of Florida; Fred W. Weingarten, American Library Association, Washington, DC.

Linguistics and Language Science
Chair-Elect: Patricia K. Kuhl, Univ. of Washington; Richard P. Meier, Univ. of Texas, Austin.
Member-at-Large of the Section Committee: Barbara Abbott, Michigan State Univ.; Richard T. Oehrle, Cataphora, Inc., Redwood City, CA.
Electorate Nominating Committee: Jeanette K. Gundel, Univ. of Minnesota, Minneapolis; Allard Jongman, Univ. of Kansas, Lawrence; Catherine O. Ringen, Univ. of Iowa; Douglas H. Whalen, Haskins Labs., New Haven, CT.

Mathematics
Chair-Elect: Carl Pomerance, Dartmouth College; Lawrence Sirovich, Mt. Sinai School of Medicine, New York City.
Member-at-Large of the Section Committee: Rick Chartrand, Los Alamos National Lab.; David Isaacson, Rensselaer Polytechnic Institute.
Electorate Nominating Committee: Edward F. Aboufadel, Grand Valley State Univ., Allendale, MI; Sheldon Axler, San Francisco State Univ.; Sandy Grabiner, Pomona College, Claremont, CA; T. Christine Stevens, Saint Louis Univ.

Medical Sciences
Chair-Elect: Jay A. Berzofsky, National Cancer Institute; Louise C. Strong, Univ. of Texas/M. D. Anderson Cancer Center, Houston.
Member-at-Large of the Section Committee: Judy Lieberman, Harvard Medical School; William E. Paul, National Institute of Allergy and Infectious Diseases.
Electorate Nominating Committee: H. Kim Bottomly, Yale Univ.; Fred D. Finkelman, Univ. of Cincinnati; Barry S. Handwerger, Univ. of Maryland, Baltimore; Phillip Scott, Univ. of Pennsylvania.
Council Delegates: Gail A. Bishop, Univ. of Iowa; Carol D. Blair, Colorado State Univ.; Michael Croft, La Jolla Institute for Allergy and Immunology, San Diego; Douglas R. Green, St. Jude Children's Research Hospital; Ashley T. Haase, Univ. of Minnesota, Minneapolis; J. Marie Hardwick, Johns Hopkins Univ.; Martin Hauer-Jensen, Univ. of Arkansas for Medical Sciences; Paul A. Insel, Univ. of California, San Diego; John S. Lazo, Univ. of Pittsburgh; Gary J. Nabel, National Institute of Allergy and Infectious Diseases; Ralph M. Steinman, Rockefeller Univ.; Joann B. Sweasy, Yale Univ.

Neuroscience
Chair-Elect: Mary E. Hatten, Rockefeller Univ.; Richard Lewis Huganir, Johns Hopkins Univ. and Howard Hughes Medical Institute.
Member-at-Large of the Section Committee: William C. Mobley, Stanford Univ.; Leslie P. Tolbert, Univ. of Arizona.
Electorate Nominating Committee: Steven Burden, New York Univ.; Gail Mandel, State Univ. of New York, Stony Brook; Earl K. Miller, Massachusetts Institute of Technology; Robert H. Wurtz, National Eye Institute.

Pharmaceutical Sciences
Chair-Elect: Margaret O. James, Univ. of Florida; Patrick J. Sinko, Rutgers Univ.
Member-at-Large of the Section Committee: Robert P. Hanzlik, Univ. of Kansas, Lawrence; Ping I. Lee, Univ. of Toronto.
Electorate Nominating Committee: William F. Elmquist, Univ. of Minnesota, Minneapolis; Anthony J. Hickey, Univ. of North Carolina, Chapel Hill; Terrance J. Kavanagh, Univ. of Washington; Dhiren R. Thakker, Univ. of North Carolina, Chapel Hill.

Physics
Chair-Elect: Lawrence M. Krauss, Case Western Reserve Univ.; Berndt Mueller, Duke Univ.
Member-at-Large of the Section Committee: Robert H. Austin, Princeton Univ.; Peter D. Barnes, Los Alamos National Lab.
Electorate Nominating Committee: Dawn A. Bonnell, Univ. of Pennsylvania; Leonard C. Feldman, Vanderbilt Univ.; Paul D. Grannis, State Univ. of New York, Stony Brook; Arthur F. Hebard, Univ. of Florida.

Psychology
Chair-Elect: Emanuel Donchin, Univ. of South Florida; Lynn Nadel, Univ. of Arizona.
Member-at-Large of the Section Committee: Morton Ann Gernsbacher, Univ. of Wisconsin, Madison; Roberta L. Klatzky, Carnegie Mellon Univ.
Electorate Nominating Committee: Jeffrey R. Alberts, Indiana Univ., Bloomington; Richard J. Davidson, Univ. of Wisconsin, Madison; Barbara L. Finlay, Cornell Univ.; Arthur S. Reber, Brooklyn College, CUNY.
Council Delegate: S. Marc Breedlove, Michigan State Univ.; Robert A. Rescorla, Univ. of Pennsylvania.

Social, Economic, and Political Sciences
Chair-Elect: Ronald F. Abler, International Geographical Union, Arlington, VA; Linda G. Martin, Institute of Medicine, Washington, DC.
Member-at-Large of the Section Committee: Ronald R. Rindfuss, Univ. of North Carolina, Chapel Hill; Jeffrey D. Sachs, Columbia Univ.
Electorate Nominating Committee: Michael Brintnall, American Political Science Association, Washington, DC; Thomas F. Gieryn, Indiana Univ., Bloomington; Patrick D. Nolan, Univ. of South Carolina; Beth J. Soldo, Univ. of Pennsylvania.
Council Delegate: Michael D. Intriligator, Univ. of California, Los Angeles; Charles F. Manski, Northwestern Univ.

Societal Impacts of Science and Engineering
Chair-Elect: Susan Hackwood, California Council on Science and Technology, Riverside; Richard B. Norgaard, Univ. of California, Berkeley.
Member-at-Large of the Section Committee: Elizabeth Chornesky, Carmel, CA; Gene I. Rochlin, Univ. of California, Berkeley.
Electorate Nominating Committee: Thomas Dietz, Michigan State Univ.; Donna Gerardi Riordan, California Council on Science and Technology, Riverside; Paul C. Stern, National Research Council; Albert H. Teich, AAAS.

Statistics
Chair-Elect: Ron Brookmeyer, Johns Hopkins Univ.; Donald Guthrie, Bainbridge Island, WA.
Member-at-Large of the Section Committee: Edward L. Korn, National Cancer Institute; Juliet Popper Shaffer, Univ. of California, Berkeley.
Electorate Nominating Committee: Emery N. Brown, Massachusetts General Hospital and Harvard Medical School; Beth Gladen, National Institute of Environmental Health Sciences; Carol K. Redmond, Univ. of Pittsburgh; James L. Rosenberger, Pennsylvania State Univ.

Q AAAs

Who's helping build the future of science?

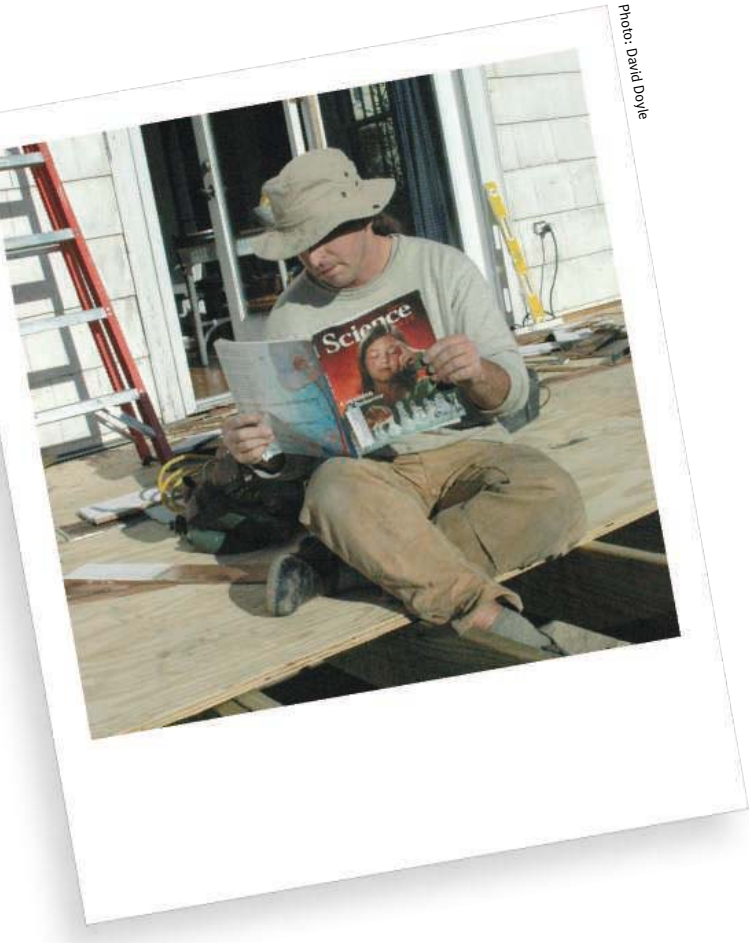


Photo: David Doyle

"I read my *Science* on the work site. Formerly a chemist, I found my true calling in woodworking, but I still try to keep up with advances in science. Reading *Science* also helps me answer questions from colleagues in the building trades about the safety and efficacy of the diverse materials we encounter."

Milton Trimitsis, carpenter and AAAS member

AAAS is committed to advancing science and giving a voice to scientists around the world. We work to improve science education, promote a sound science policy, and support human rights.

Helping our members stay abreast of their field is a key priority for AAAS. One way we do this is through *Science*, which features all the latest breakthroughs and groundbreaking research, and keeps scientists connected wherever they happen to be. Members like Milton find it essential reading.

To join the international family of science, go to www.aaas.org/join.

To see other member photos, please visit:
http://promo.aaas.org/memberpics.shtml

Milton Trimitsis

AAAS
ADVANCING SCIENCE, SERVING SOCIETY

www.aaas.org/join

INTRODUCTION

Inside the Pipeline: Pharma Goes to Work

An old joke about nuclear fusion research is that the technology for generating a cheap and plentiful supply of energy has been "just over the horizon" for the past 50 years. The drug industry is the focus of similar optimism but with a shorter time frame: Its research pipeline is invariably described as being on the verge of delivering novel and powerful treatments for dread diseases.

Unfortunately, that pipeline has a chronic leak that no pharma executive has been able to plug. "What hasn't changed in the past 25 years is that only one of five compounds that enter clinical trials comes out as a product. That's amazing to me," says Robert Gorman, former head of worldwide drug discovery at Upjohn-Pharmacia, who retired before the company was acquired by Pfizer in 2003. "I spent hundreds of millions of dollars on databases and other tools to try and improve that performance."

What could the industry do to reduce the size of the leak? The answer is complicated. As the eight stories in this package and related articles in two of *Science*'s online publications, the Signal Transduction Knowledge Environment (www.stke.org) and Next Wave (www.nextwave.org), make abundantly clear, the process of discovering new drugs is fiendishly complex, vastly expensive, and wildly unpredictable. Some of the factors are beyond anyone's control: Researchers are working at the frontiers of science, combining knowledge with educated guesses, and their companies are betting millions on untested compounds only to watch most of them crash and burn. Measuring inputs and predicting outcomes is a game that every research honcho plays but with limited success.

At the same time, some of the industry's problems appear to be at least partially self-inflicted. The wave of mergers and acquisitions in the past decade has had a debilitating effect on morale and productivity, according to some analyses. Within companies, marketing departments are said to exert an undue influence at times on scientific decisions about which projects move through the lab and into clinical trials.

PAGE 731

Training also seems suboptimal. Industry scientists typically learn on the job, not in school. "No university teaches drug discovery," says Graeme Bilbe, head of global neuroscience for Novartis. "And many don't give much credence to what we do." At the management level, many pharma executives who joined industry after successful academic careers freely admit that they were shockingly naïve about what it takes to make a drug. "Even if you were thought of as a superstar in academia, the chances are that you don't really know how things work in industry," says Ben Shapiro, a retired research executive at Merck, who left the University of Washington in midcareer. "And if you're deluded enough to think that you do understand, you'll run into trouble."

For all its problems, however, the drug industry appeals to thousands of scientists hoping for a chance, against long odds, to improve the lot of humanity. "I was considered a sellout in 1980 when I went to work for Merck," recalls Amgen's Paul Reider. But he never regretted his decision, and over the next decade he helped the company develop Crixivan, the first protease inhibitor to treat AIDS patients. "It's hard to give up on a drug," he says, "if you know that thousands of people will die without it."

—JEFFREY MERVIS

CONTENTS

NEWS

See also Editorial on p. 669 and www.sciencemag.org/sciext/drugdisc05

NEWS

The Hunt for a New Drug: Five Views From the Inside

The world of drug discovery in big pharma can seem pretty mysterious to outsiders. But some patterns are visible from the inside

Waiting for his lunch to arrive, Graeme Bilbe wants to make sure that the reporter on the other end of his cell phone understands how hard it is to discover a new drug. The U.K.–born, Basel, Switzerland–based head of global neuroscience research at Novartis is dining in southern California with a former pharma colleague, Tamas Bartfai, now chair of the department of neuropharmacology at the Scripps Research Institute in La Jolla, California. The hors d'oeuvre is a lecture on the industry's staggering attrition rates.

"How many ideas do you think you need [to develop a drug]?" demanded Bilbe, who's been with Novartis since 1989. "Take a guess. One thousand? Ten thousand? You need at least that many, if not more. The chances that any of those ideas will ever become a drug are vanishingly small."

Those mind-boggling numbers color everything about research in big pharma and make this research sector distinct from any other area of industrial research. Very few pharma scientists actually work on products. Instead, the vast majority toil at a much more basic level, looking for potential targets, synthesizing compounds that might act on those targets in a way that would be therapeutic, and then making the compound "druggable." "I have never worked on a successful drug," confesses Derek Lowe, a medicinal chemist with 16 years in the industry who writes what may be the only Web log (blog) dedicated to pharmaceutical research (www.corante.com/pipeline). "Heck, I haven't worked on anything that anybody with a disease has ever put in their mouths."

The research environment has also been reshaped dramatically in the past decade or so by mergers, which can abruptly shift a researcher's focus onto a whole new area of study. And unlike research on a new computer chip or a more efficient engine, the output from pharma research labs is not so easy to measure (see p. 726).

The world of big pharma research is shrouded in a culture of secrecy that goes well beyond the specific compounds and targets a company is working on. Here's how an otherwise candid Lex Van der Ploeg, head of Merck's new research lab in Boston (see sidebar, p. 723), puts it when asked about his productivity goals. "If I told you that this lab was going to generate, say, eight lead candidates this year, then our competitors could look at the number of people we employ and figure out how many people it takes us to develop a candidate compound," he says. "Then they would compare it to how many it takes them. And if we're lower, they'd try to figure out why, and what they can do to become more efficient. That would give them a competitive advantage."

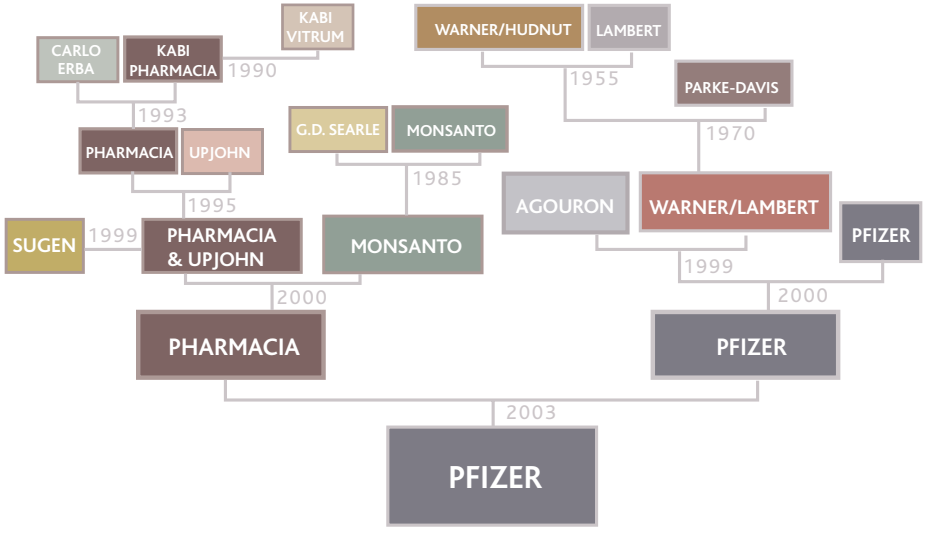
Knowing where they stand is an all-consuming interest for pharma executives. As a result, investment analysts and corporate consultants churn out reams of reports each year on industry trends, from early-stage alliances with biotech companies possessing intriguing compounds to the latest technology "platforms" that can improve efficiency. The documents are sprinkled liberally with breathless predictions about how these trends "will change everything."

Most of the time they don't, of course. In the meantime, however, these big-picture studies provide little idea of what the view is like from inside the industry's labs. From the scores of scientists and research managers we interviewed for this special section, we have chosen five individuals whose stories provide glimpses of how those big trends trickle down to the labs and computer workstations around the globe that represent ground zero in the hunt for new drugs.

A view from the bench: Change as a constant

Eric Gulve joined big pharma in 1993 in hopes of ameliorating the ravages of diabetes. A research assistant professor at Washington University in suburban St. Louis, Missouri, Gulve became part of a team at G. D. Searle (the pharmaceutical arm of Monsanto) that was just beginning to tackle insulin resistance in type 2 diabetes. Since then, he's worked on cholesterol metabolism for Monsanto, cardiovascular diseases for Pharmacia, and then diabetes again for Pharmacia. Today he's with Pfizer, seeking potential targets to treat two forms of cardiovascular disease, thrombosis and hypertension.

Although he's worked for three companies in 12 years, Gulve is no hired gun. He hasn't even changed his commute to work. Rather, the 46-year-old physiologist has spent his entire pharma career in the same four-story industrial lab in the St. Louis suburb of Creve Coeur. The job changes were the result of three corporate mergers, culminating in Pfizer's $53 billion acquisition of Pharmacia in April 2002. Those mergers triggered top-down reviews of existing research, followed by projects or entire areas of therapeutic research being cancelled or



Growth industry. Today's Pfizer is built upon a decade and more of dealmaking.

CREDITS (TOP TO BOTTOM): STEVE WAGNER; PFIZER

Boston Means Business for Drug Companies

BOSTON, MASSACHUSETTS—Asked why he robbed banks, Willie Sutton is said to have responded: because that's where the money is. After more than a century, big pharma is following that logic by setting up shop here amid what may be the world's largest concentration of biological brainpower.

Two of the world's biggest drug companies, New Jersey–based Merck and Connecticut-based Pfizer, have opened small outposts to supplement their global R&D networks and put company turnaround artists in charge of them. A third pharma, Novartis, has gone even further by relocating its main research facility, the Novartis Institutes for Biomedical Research (NIBR), in a spectacularly remodeled former candy factory and two other buildings adjacent to the Massachusetts Institute of Technology (MIT) and picking an industry novice to run it. The 1000-strong scientific work force assembled in the past 2 years represents a serious bid by the Swiss-based company to find the sweet spot in drug discovery.

"Our kickoff career fair attracted more than 2000 people, and it was a fabulous opportunity to meet and greet leading scientists and business leaders," says Lynne Cannon, vice president for human resources at NIBR. "That would have been difficult to do in Groton [Connecticut, the site of Pfizer's largest lab] or Princeton, New Jersey."

Boston may be the cradle of American independence, and Cambridge the home of the country's oldest and most prestigious university, but until the past few years the region wasn't even on the map of big pharma. Area academics with backgrounds in molecular biology had formed many biotechnology companies, some of which aspired to become the next big pharma. However, the nation's chemical-based drug industry was confined to the mid-Atlantic region and the Midwest.

Pfizer made the first move in 1999, opening up a Discovery Technology Center in Cambridge that offered the latest technology to drug discovery scientists throughout the company. Last year, officials expanded the center's mission to the entire pipeline of drug development and plucked Phil Vickers from the company's ranks to run it. A 45-year-old biochemist who enjoys a challenge and a change in scenery, Vickers was born in England, received his Ph.D. at the University of Toronto, and did a postdoc at the National Institutes of Health in Maryland before joining Merck's Frosst laboratory in Montreal in 1988. He came to Pfizer in 1994 and earned his stripes in a series of management posts on both sides of the Atlantic.

Perched on the edge of the MIT campus, the renamed Research Technology Center aims to satisfy Pfizer's need for technological support by mixing in-house expertise with the skills of local academics and start-up companies. Vickers says his youthful but growing shop—he plans to add 25 scientists to the current 110-person roster by the end of the year—"offers the attributes of a biotech with the resources of a big pharma."

Despite running an operation almost 10 times the size of Pfizer's, Mark Fishman describes NIBR in similar terms. A molecular cardiologist who had pioneered the use of zebrafish for gene discovery at Harvard Medical School (HMS) and Massachusetts General Hospital, Fishman is hoping to "functionalize the genome" by applying it to diseases where the biological mechanism is already understood. The lab's location—the region has supplied more than half the institute's talent, not to mention an ever-widening network of academic collaborations—provides an added boost, he says.

Already, Fishman has raided HMS to find global heads in cardiovascular research and modeling disease. He's also tapped biotech and pharma for chiefs in oncology, molecular pathways, and discovery chemistry, luring them with the prospect of painting on a fresh canvas. "We're getting who we want, and almost nobody has left," he crows.

Candyland. Novartis converted a candy factory for its main research institute.

Across the Charles River and adjacent to Boston's medical complex sits Merck's Edward M. Scolnick Research Laboratory. Named in honor of its former research chief, the new 12-story, glass-faced lab opened last fall, and its site head, Lex Van der Ploeg, is busily recruiting talent. Van der Ploeg, 50, a specialist in infectious diseases who joined Merck in 1991, took on the challenge after a year spent shifting the focus of Merck's San Diego facility from neuroscience to stroke. Soon after he left, however, corporate officials decided to shut the lab and shift some resources to other sites.

His mission is to rev up the company's efforts in developing treatments for cancer, obesity, and Alzheimer's disease. He expects to double the size of the basic research team, now 140, by 2007, beginning with oncology and then moving into the neurosciences. "The proximity to talent is terrific, and our success rate is about 90%," he says about current recruiting efforts. About a quarter of the scientists have migrated from other Merck labs.

–J.D.M.

transferred to another site. During one gut-wrenching transition, Gulve spent weeks interviewing scientists for a revamped department—without knowing whether he would be their boss or even if he would still have a job with the new company.

There's no way to know if Gulve's career path is typical. Some scientists remain at one company their entire lives, and others switch jobs often and voluntarily. But mergers have clearly changed the landscape of big pharma in the past decade. Gulve's current employer, with $52 billion in sales last year, has become the industry's leader thanks to its ingestion of Warner-Lambert in 2000 and Pharmacia, each of which in recent years had swallowed smaller fish such as Parke-Davis, Upjohn, Monsanto, and G. D. Searle.

"I'm not complaining about any of the decisions that were made," he says. "But it is frustrating when you've worked so long and hard on a project and still haven't gotten far enough along to know if your hypothesis is right or wrong. I know that mergers are part of the business. But I hope that I never have to go through another one."

A view from a loyal critic: The art of drugmaking

Derek Lowe may be unique in the pharmaceutical industry: He's a medicinal chemist for a big pharma who writes a blog on drug discovery. His column (www.corante.com/pipeline) is an irreverent look at the industry. It's filled with pinpricking commentaries on the latest clinical results, corporate reshufflings, and

CREDIT: NOVARTIS

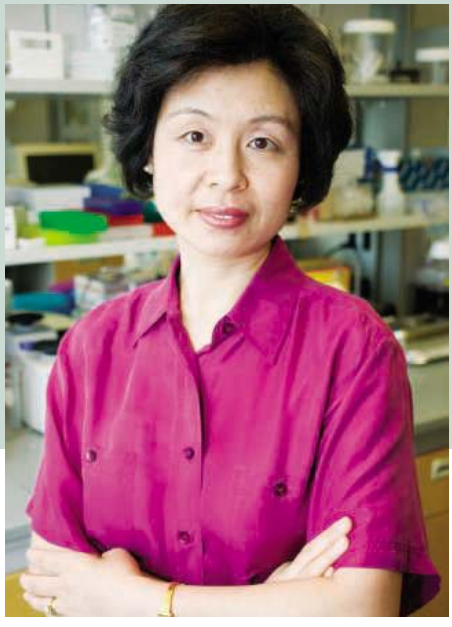
It's Still a Man's World at the Top of Big Pharma Research

For a few years after their company was acquired by Wyeth in 1995, molecular biologist Abbie Celniker and several female colleagues at Genetics Institute in Cambridge, Massachusetts, hoped that the new management might boost their careers. But eventually they came to the opposite conclusion. "There was an established culture [at Wyeth] that said it would be harder to influence our peers. ... Simply put, we didn't see a career progression unless we learned to play golf and use the men's room."

What Celniker, now senior vice president for strategic research at Millennium Pharmaceuticals in Cambridge, had sensed becomes obvious by looking at the leadership rosters of the research divisions of big pharma: Drug discovery is a man's world. Not one of the chief scientists or heads of research at these companies is a woman. The precious few senior women executives with science Ph.D.s or M.D.s are most often found on the development/business side of the company or holding corporate posts without line responsibilities.

Why that's the case, however, is much less clear. Ask a man and you're likely to hear that the industry is no different from the rest of society. Then he'll note that his company is very concerned. "It's a tough issue that I think about a lot," says Jonathan Knowles, head of global research for Roche. "I'd like to understand it better." He'll also say that things are getting better.

Ask a woman—who by definition has not made it to the top—and her answer will be quite different, although equally nuanced. "The forces keeping women scientists down are more psychological and cultural than legal," says Joanne Kamens, a project team leader at Abbott Bioresearch Center in Worcester, Massachusetts, and president of the state chapter of the Association for Women in Science. "People still have a problem seeing women as leaders rather than as caretakers and mothers. Men who decide to spend more time with their families also tend to be seen as weaker. But at least they have the option. If the father can't help out at home, it falls on the women."



Barrier-free. Novartis's Lijun Wu makes room for both career and family.

CREDIT: EVAN BURROWS/NOVARTIS

overhyped trends in the business. He's not embarrassed to describe his own failures, either, including an on-again, off-again attempt to test a hypothesis that stubbornly resists verification.

His daily musings generate 25,000 hits a month. That traffic feeds Lowe's need for an audience, a hunger that offsets the lack of payment for his labors. "It hasn't helped my research," he confesses about the blog, which he started in 2002. "But it's given me a much broader perspective on the business." His readers are both colleagues—"insiders write me about how they've tried the same things in their labs that I write about"—and outsiders with a voyeuristic streak. "Where else would I get to hear from people saying, 'When I took that drug you wrote about … ' I've also done some historical reading about the fashions that sweep through the industry and the fact that most of them don't pan out."

A 1988 chemistry Ph.D. from Duke University, Lowe wanted to teach at a small liberal arts college but couldn't find the right job. Answering a job ad has led to a career in industry that he says "has worked out pretty well." He currently works for Bayer but goes to great lengths to separate his dual identities as a researcher and blogger.

Lowe doesn't hesitate to point out the foibles of the pharmaceutical industry. "We're not angels. And when we mess up, I say so. If I was rah-rah all the time, nobody would read me."

Even so, he's as dedicated to improving human health through modern drug discovery as any pharma bigwig. Taking umbrage at a recent story in *Business Week* entitled "Biotech, At Last" that paints academic research as nimble and pharma science as hidebound, Lowe writes: "It's true that many of the basic discoveries that have led to the current biotechnology industry came from academic research. That's just as it should be. But none of it would have been turned into human therapies without corporate research and development." And he's personally offended at the article's characterization of pharma's relationship with biotech in the 1980s and 1990s. "*Shied away* from biotech for years? We pumped uncountable billions into it, much of which we never saw again."

A career-eye view: A taste of industry

What's a postdoc doing in pharma? Scottish-born David Dornan has spent nearly 3 years at Genentech, which has a 30-year-old policy of seeking out promising young scientists to pursue basic research. And although the company has a rule that its postdocs don't move into permanent positions, Dornan sounds like someone whose career aspirations may have been altered by working at the South San Francisco, California, biotech giant.

"My future? I think of it every day," says the 27-year-old Dornan, who earned his Ph.D. in molecular oncology at the University of Dundee, U.K. "And the longer I'm here, the more difficult it is to envision becoming an academic."

A member of a team led by Genentech's head of oncology V. M. Dixit, Dornan was a co-author of papers in *Science* and *Nature* last year that describe the group's work on how cancer-related proteins are degraded by the ubiquitin system. And although the work is fundamental science, Dornan has also been bitten by the drug discovery bug. "We found something that could be a therapeutic, and we have a unique chance to put it into development. It depends on the next phase. And if it works, we'll be handing it off to the chemists. The point is that it's possible."

Dornan is realistic about his chances of staying on the West Coast. "California is great, but you have to be willing to go where there's a job."

A view from a distance: Landing on her feet

When Myrlene Staten saw the job ad in the *New England Journal of Medicine* in 1989, she thought it could have been written just for her. "Roche wanted a junior faculty member with clinical experience in metabolic diseases," she recalls. Her work as an endocrinologist at Washington University in St. Louis made her a perfect fit, she realized, and before long she had moved from Missouri to New Jersey to help the company develop drugs for obesity and diabetes.

It was the start of a 15-year odyssey through big pharma that she recalls with mixed emotions from her current post at the National Institute of Diabetes and Digestive and Kidney Diseases in Bethesda, Maryland, where she runs a program to encourage academics and small companies to develop new therapies for type 1 diabetes. After 4 years at Roche, she moved to Lederle, which was soon bought by Wyeth. In 1995, she headed out west to Amgen, where she was part of the team doing

Lijun Wu, a 41-year-old unit head within the cardiovascular group at the flagship Novartis Institute for Basic Research (NIBR) in Cambridge, Massachusetts, remembers being asked as a graduate student if her decision to get married meant that she planned to drop out of the program to have a family. Several years later, after becoming pregnant with the first of her two children, colleagues told her that her bosses at Millennium were wondering if she'd return after giving birth. "My career was going well, and they didn't ask me directly. But I think it's unfair; they wouldn't have wondered that about a man."

Wu doesn't understand why any employer would care whether she even has a family. But most pharma executives acknowledge that family responsibilities do matter. "One possible reason [for the dearth of women] is that any senior position requires a huge commitment," says Knowles. "It would be difficult for someone to do that type of job while also looking after a home and small children."

Amgen's research chief Richard Perlmutter offers similar thoughts. "I'm reluctant to generalize about gender differences," he says. "At the same time, you can't get around the fact that the burden of early child rearing may be a career breaker [for some women]."

That burden can show up in subtle ways, notes Lynne Cannon, vice president for human resources at NIBR. "It's not just a question of having the door open to women," she says. "Sometimes it's about how the door gets opened. If I can't stay until 8 p.m.—when a lot of decisions get made—because I have to pick up my kid at 6 from daycare, then I may miss out on something important."

Many pharma companies have recently begun to identify and assist women scientists who want to move up the corporate ladder. Novartis has a "women to watch within the lab" program, Cannon says, to provide ongoing career guidance and support for outstanding women. "Mentoring is great," says Cannon, "but there's a danger if you attach yourself to one person and that person leaves." Although that's true for men, too, the dearth of women makes any loss of support costly.

Wyeth has a similar program for top-performing women, says Robert Ruffolo, president of research and development, that's modeled on a gender-blind program for the top 1% of its researchers. Gail Cassell, vice president for strategic planning for Eli Lilly, says that the Indianapolis, Indiana–based company offers a variety of programs for women scientists, from tips on how to ask for a promotion to networking with colleagues in other fields.

None of the programs has run long enough to accumulate meaningful data, however. And it's not clear that company executives have thought in much detail about what they want to achieve. "We don't know what enough is," Ruffolo admits. "But we consider it a win as long as we're attracting more women and minorities each year than are leaving the company."

–J.D.M.

the company's first clinical trials on the protein leptin, once highly regarded as a potential diet drug. Then it was back to the East Coast for a 2-year stint with Bristol-Myers Squibb before joining Upjohn/Pharmacia, where she was head of metabolic diseases.

A 2000 merger with Searle resulted in a spinoff of the new company's metabolic diseases portfolio to a new biotech based in Stockholm, Sweden. But Staten found a way to stay in New Jersey. "Searle had a cardiovascular group, and it had an opening. So when the boss called me in and asked me how I felt about working on cardiovascular diseases, I said, 'Real good.' "

Two years later, Staten had to handle another merger, this one with Pfizer. After helping the new company analyze its combined portfolio—"you present your project to senior managers, who then go behind closed doors and come out months later with a list of what stays and what goes"—she was faced with finding a new position. Choosing a very different direction, Staten jumped back into the nonprofit world, landing at the National Institutes of Health.

She's lost none of her zeal for finding new medicines that can help people. But she's 15 years wiser about how difficult that is, and how failure is a much more likely option. "My goal, then and now, is to develop a drug that can achieve a 20% permanent weight loss with no unusual side effects. But I think maybe I'll have to leave that to the next generation."

A view from the executive office: Finding his niche

At 54, Bob Stein is a drug industry veteran who has done it all, including two stints with biotech. After several coast-to-coast moves, he says he's exactly where he wants to be: president of Roche's research lab in Palo Alto, California.

A graduate of a joint M.D./Ph.D. program at Duke University in Durham, North Carolina, Stein came to Merck in 1981 along with Edward Scolnick, who later became the company's legendary research chief. (Scolnick had originally offered him a job at the National Cancer Institute in Bethesda, Maryland, but then signed on at Merck before Stein said yes.) Stein says he found the company's scientific environment "much more exciting" than academic jobs he had been offered. Several years later, as head of pharmacology at Merck, "where I worked on some pretty good drugs," he was dispatched one day to San Diego, California, to assess a possible collaboration with a company, Ligand Pharmaceuticals, that "had great science but no infrastructure for drug discovery." After recommending that Merck walk away from the deal, Stein was headhunted to become Ligand's chief scientific officer.

CREDIT: COURTESY OF BOB STEIN

All for one. Drug discovery puts a premium on teamwork, says Roche's Bob Stein.

Within a year, Ligand had raised $250 million in a public offering, and Stein had negotiated eight collaborations with big pharma. But the grueling schedule—including talks at 13 investment meetings and 250 business presentations—and the amount of work it took to "get other people to do what needed to be done" at the small company led him to embrace an offer from a mentor to return to East Coast pharma. The job was as head of research and preclinical development at DuPont Merck Pharmaceuticals, a joint venture of the two companies.

"I'd have been happy to stay there, too," Stein says about his 6 years there. But DuPont decided that the joint venture was chewing up too much of its research budget, he says, and after Bristol-Myers Squibb bought the company for $7.8 billion, "I didn't like what I saw." So he jumped to Incyte Corp., where he spent 2 years as president and chief scientific officer before joining Roche in 2003.

Stein clearly loves the horsepower of a big pharma and enjoys the chance to apply what he's learned over a quarter-century about drug discovery. But greater capacity means a greater chance to fail, too. "The goal is to develop superior medicine," he says. "But the process includes a million handoffs, and any dropping of the ball could be potentially devastating."

–JEFFREY MERVIS

NEWS

Productivity Counts—But the Definition Is Key

With costs soaring, every company says it's becoming more efficient. But what exactly does that mean?

For all but a tiny fraction of big pharma scientists, their work isn't really about discovering new drugs to cure disease and improve human health. It's about looking for druggable compounds: molecules that might bind to targets that could block or enhance a biochemical process that leads to a particular pathological state or impairment. And success isn't measured by how much they have contributed to a drug or therapeutic medicine on the market. Rather, it means "hitting your numbers," that is, achieving a preset goal of "deliverables"—be they compounds, animal data, or patients—that argue for moving along to the next step in the process.

Trouble is, that approach is hugely inefficient. The current cost of discovering and developing a new drug may be as high as $1.9 billion, according to an extrapolation by Joseph DiMasi of the Tufts University Center for the Study of Drug Development in Boston, Massachusetts, whose 2001 report pegging the number at $802 million was based on medicines that entered clinical trials as long as 20 years ago. Lowering that number is the current Holy Grail of the industry. "Productivity is our biggest challenge and the number one topic of conversation among my colleagues," says Steven Paul, president of Lilly Research Laboratories and the top scientist at the Indianapolis, Indiana–based drug giant.

But consensus on the goal doesn't mean agreement on how to get there. Big pharma management features a multiplicity of organizational models, all aimed at achieving greater efficiency. Some companies such as Pfizer are highly centralized, whereas others pride themselves on having small, semi-autonomous units. "Pfizer is probably at one end of the spectrum. Everything related to drug discovery has to go through either New London [Connecticut] or Sandwich, U.K.," says industry analyst Roger Longman, co–managing partner of Windhover Information Inc. in Norwalk, Connecticut.

At the other end, he notes, is U.K.-based GlaxoSmithKline (GSK), second in global pharmaceutical sales to Pfizer. Under the leadership of research chief Tachi Yamada, GSK has created Centers of Excellence in Drug Discovery around the world in six therapeutic areas, plus one center for biologics. Each has its own budget and hiring authority. "I wanted them to be small, and studies show that you can know the names of 300 people but no more," says Yamada. The centers "have total control of their budgets and hiring. But they still have targets."

Falling somewhere in the middle is a "hub-and-spokes" system that Roche follows that allows its corporate headquarters in Basel, Switzerland, to keep tabs on research sites in the United States, Europe, and China. And although that arrangement can mean 2 a.m. teleconferences for Bob Stein, who oversees 1100 people at Roche Palo Alto, California, he says it's vastly preferable to having "one big R&D operation that, like a 10-foot spider, has outgrown its body plan."

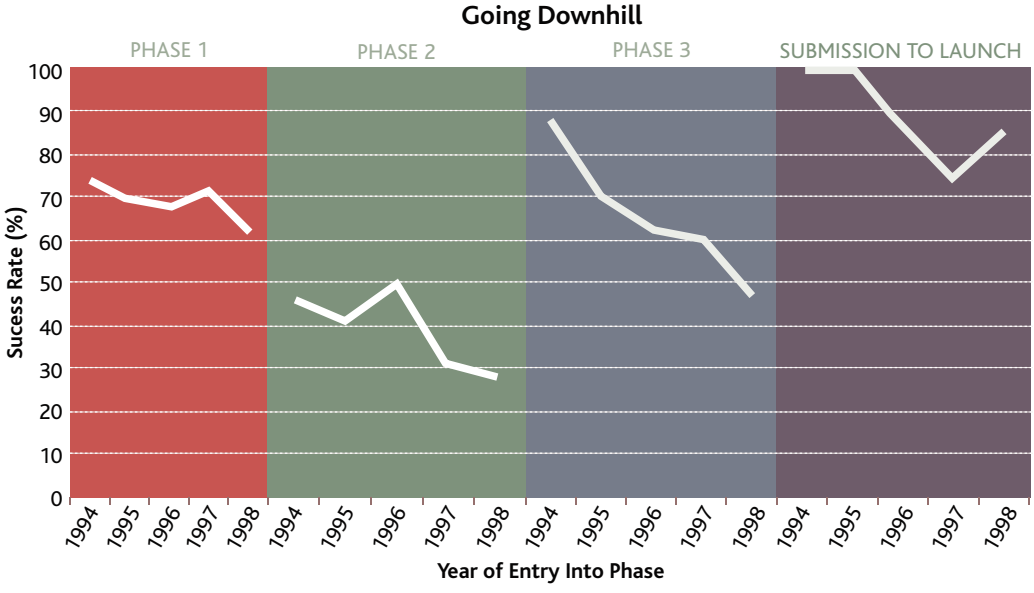
There are also many views on which metrics are the most meaningful, and if metrics can even take you where you want to go. One popular view, espoused by Pfizer CEO Hank McKinnell and others, embraces "shots on goal." That's the belief that more compounds going into clinical trials translates into more successful outcomes and, ultimately, more marketable drugs.

But what kind of shots are most important? For Yamada, the key metric "is not the number of targets validated, or the number of chemicals selected. It's proof-of-concept in patients." His counterpart at Novartis, Mark Fishman, puts it even more bluntly. "[A drug candidate] is not a success until we've treated a patient with it."

At New Jersey–based Wyeth Pharmaceuticals, which sits on the centralized end of the management spectrum, R&D president Robert Ruffolo has done a scientific analysis of the science of drug development. A 55-year-old pharmacologist and 28-year industry veteran, Ruffolo likes to say that "we've got numbers on everything." And since coming to Wyeth in 2000, Ruffolo has probably gone further than any other pharma honcho in trying to quantify what his researchers should accomplish at each stage of the process.

"Some people say that they can pick winners," Ruffolo told a meeting of pharma scientists gathered this spring in Washington, D.C. "But I believe that it's still a crapshoot. I can't pick winners, and after 30 years in this business, I haven't met anybody who could."

What Ruffolo can do, he says, is ride herd on the factors that he can control. Hence his insistence on production targets that take attrition into account and, if met, would allow for a sufficient flow of new compounds through the pipeline. Raises are based on achieving the goals, and it's all computerized.



The wrong direction. The industry's overall record of success in testing drugs in humans has declined in recent years across each step of the process.

SOURCE: CMR INTERNATIONAL

The magic numbers for Ruffolo are 12, 8, and 2. That's a three-link chain of the annual number of compounds entering development, the number of investigational new drugs entering clinical trials each year, and the annual number of new drug applications submitted to the U.S. Food and Drug Administration. He says that his approach has helped turn around what he calls the company's "pathetic" track record of submitting new drug applications in the years before he arrived. And best of all, it's proven to be sustainable: Wyeth has met the targets every year since 2001, he says. "That's the most important point. It's a steady-state model."

Ruffolo admits that approach didn't win him any popularity contests at Wyeth. "Scientists hate this approach," he says. "When I was a scientist, we used to say that you can't manage science. But it needs to be." Those who didn't buy into the approach left the company, he says—and those who have remained appreciate knowing where they stand.

Richard Scheller takes a very different approach as executive vice president of research at Genentech, which has eschewed large acquisitions and does all research at its ever-expanding South San Francisco, California, campus. A neuroscientist and former Howard Hughes Medical Institute investigator at Stanford University, Scheller came to Genentech in 2001 after deciding that its culture meshed with his own philosophy of doing science. Genentech's corporate strategy, labeled Horizon 2010, does include research goals for its more than 600 scientists over the next 5 years. But although they specify the number of new products to be moved forward for each of the company's three major therapeutic areas, some goals omit key steps in the process. And they aren't linked together in a formal manner.

Sitting in a top-floor office overlooking San Francisco Bay—and the pier that was allegedly the favorite fishing hole of cofounder Herbert Boyer—Scheller describes an ongoing study of Genentech's attrition rate and the nature of its pipeline in a way that suggests he doesn't view it as quite the priority that Ruffolo does. "It turns out that different types of projects fail for different reasons," notes Scheller, who says that he "doesn't know very much about big pharma" despite the fact that, based on the value of its stock, Genentech is the fifth-largest drug company in the world.

"For example," Scheller says, "I'm expecting small-molecule throughput rates to be lower than for protein therapeutics. I'm also leading a project to understand the bottlenecks. And I think that they will turn out to be what you'd expect: Some projects will be underresourced, some will suffer from poor internal communications. When we're finished, we'll react appropriately. But I suspect that when we fix one problem, some other bottleneck will appear."

Don't be fooled by that dispassionate tone, however. Scheller isn't afraid to be just as hard-nosed as Ruffolo in assessing the performance of his troops. But he doesn't plan to do it from a spreadsheet. Knowing how to maintain a healthy pipeline, he says, "is more or less a matter of intuition." And the most important thing about dealing with scientists, he says, "is to be clear about the reasons for your decision [for killing a project or shifting resources]. I'm not always going to be right. But I've earned a lot of respect from my credentials at Stanford and my achievements as a scientist."

—JEFFREY MERVIS

NEWS

I See You've Worked at Merck ...

Senior hires at Amgen demonstrate how one company's loss can be another's gain

In 2000, Merck CEO Raymond Gilmartin came down from New Jersey to Washington, D.C., to extol the value of research partnerships involving the government, academia, and industry. In a talk marking the centennial of the Association of American Universities, Gilmartin mentioned two executives he hoped would help the company set up a research lab in Boston (see sidebar, p. 723) to tap its rich talent pool: Roger Perlmutter, head of basic research, and Ben Shapiro, his predecessor and current head of external research. He also noted that Merck's success in the development of the first protease inhibitor to treat AIDS, Crixivan, by a team led by chemist Paul Reider, rested on the government's long commitment to basic biomedical research.

Fast-forward 5 years—a generation in big pharma—and none of the four team members still works at Merck. In May, Gilmartin suddenly stepped down earlier than expected, a casualty of the company's voluntary withdrawal last fall of Vioxx, its arthritis painkilling COX-2 inhibitor pill. Shapiro had retired in 2003, in keeping with company policy for executives who reach age 65.

Perlmutter and Reider remain very active in the drug business. But they now work for Amgen, the southern California biotech giant that industry wags have dubbed "Merck West." Their migration is illustrative of the company's role over the years as both a magnet for top academic talent and a fertile hunting ground for competitors. And even as market analysts wonder if Merck can recover

Coast to coast. Amgen's senior research team is led by former Merck scientist Roger Perlmutter (*left*) and includes several former—and current—colleagues such as Paul Reider (*right*).

CREDITS (RIGHT TO LEFT): JAMEY STILLINGS; ELTON GREIG

from the blow to its reputation from Vioxx and the financial burden of stock shares trading at one-third their 2000 level, several successful alumni say that they retain warm feelings for "Mother Merck." Several senior research officials at Merck declined comment for this story.

Shapiro, for one, thinks "it's logical that Merck would help seed the leadership ranks of other companies." In 1990, when he was a department chair at the University of Washington (UW), Shapiro says he jumped at an invitation from Ed Scolnick, the former head of Merck research, to become head of basic research because "Merck had a reputation for caring about science." In 1997, Shapiro, in turn, recruited UW immunologist Perlmutter.

While Shapiro was grooming Perlmutter for the top research job, saying: "He was special. There aren't that many academics who would be good at senior management in big pharma," Scolnick had other plans. In December 2000, he brought on Peter Kim, a biochemist at the Massachusetts Institute of Technology. Although it would be another 2 years before Scolnick retired and Kim succeeded him, the line of succession was clear. So it was no surprise that in January 2001, Perlmutter was named executive vice president of research and development at Amgen. He says that he had been weighing several career options and chose Amgen because of "the magnitude of its commitment to building up its R&D operation." He also was attracted to its relative youth—it was founded in 1979—compared with its century-plus-old pharma competitors, and its strength in biologics.

But Perlmutter wasn't turning his back on Merck. He says he had declined an offer to join Amgen in 1996 because "I wasn't ready. I didn't understand the totality of drug discovery and development enough to have the impact that I wanted to have." Merck gave him that knowledge; he says: "My experience there informs everything that I do here."

One lesson was to tap into his Merck connections. One call went to a former medical and graduate school colleague, pathologist Joseph Miletich, who had spent most of his career at Washington University in St. Louis, Missouri, before Shapiro convinced him that Merck offered "a bigger canvas." Four years and several promotions later, Miletich heard a similar recruiting pitch from Amgen, which he joined in 2002 as senior vice president for research and preclinical development.

Not long after, Perlmutter reached out to Reider, who had come to Merck in 1980 right out of graduate school on a mission to conquer dread diseases. Reider found Perlmutter's description of Amgen as an eager teenager full of promise appealing, as well as his pledge that the company would only work on treatments for important and unmet medical needs.

"I can't get very excited working on the ninth molecule to correct male impotence, or to treat male pattern baldness," says Reider, Amgen's vice president for chemistry. "I'm somebody who needs to go home every day with a sense that I've accomplished something. I'm also 53, and it would be nice if we could find a treatment for Alzheimer's by the time I need it."

Reider says that his "dream job" would be to work with both Perlmutter and Kim, whom he says he "cherishes." But he's worried that Merck could stumble and lose its way. "Amgen today is where Merck was 15 or 20 years ago. The ability to pounce on an idea and take it into development quickly is so important. Merck still has tons of good people. It would take 30 years to lose that edge. But when you get so big that your chief concern is what products to bring to market in what time frame, that's a warning sign."

—JEFFREY MERVIS

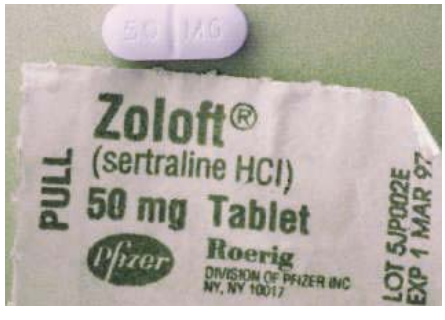
NEWS

The Brains Behind Blockbusters

The inventors of top-selling drugs talk about their unlikely paths to success, and whether today's scientists can pull off similar feats

How does a scientist hit a home run in the drug business? For Kenneth Koe and Willard Welch, it took curiosity, determination, and a series of lucky breaks. The payoff for their employer, Pfizer, was huge: the antidepressant Zoloft, one of 19 drugs that last year generated more than $2 billion in revenues in the U.S., according to IMS Health, a company that collects and markets health care data.

Koe, a biochemist, and Welch, an organic chemist, are members of a small, exclusive club of drug discoverers whose labors have helped catapult their companies into the ranks of the world's most profitable. But while aggressive advertising has made household names of drugs such as Lipitor, Nexium, and Celebrex, their inventors remain relatively unknown. *Science* interviewed nearly a dozen of them to learn the stories behind their discoveries. Although these superinventors identified very different drugs, across oceans and decades, their experiences are more similar than one might expect.



Blockbuster. Zoloft, a drug with sales of $3 billion a year, likely wouldn't exist without the work of Kenneth Koe and Willard Welch.

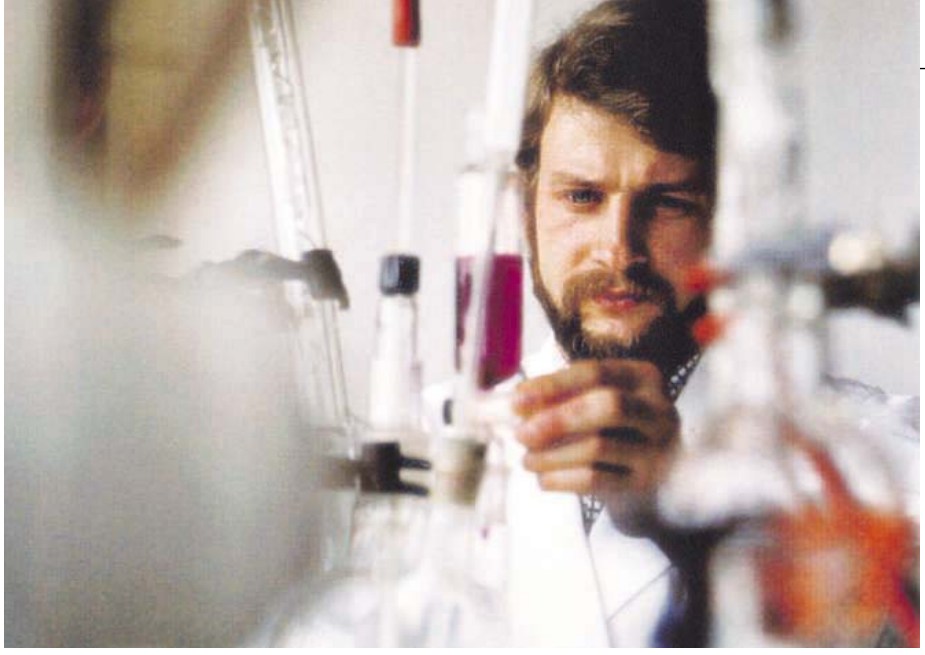
For one, few grasped the value of their discovery at the time or anticipated the hurdles standing in their way. Even fewer profited from their accomplishment, a fact that many quietly resent. Some doubt that they would be allowed to pursue the same lines of research today that they chased 15—or 40—years ago. "It was nice and unsophisticated"—and more fun—"in those days," says Bruce Roth, the Pfizer chemist who, at the tender age of 31, helped invent Lipitor in 1985.

For many scientists near or at retirement age, the advantages of today's powerful drug-hunting technologies are offset by what they see as a loss of freedom to stretch one's mind around novel ideas. "Too much computer and not enough brain," grumbles former Merck biochemist Alfred Alberts, who helped invent Mevacor, the first successful statin, as well as its $6-billion-a-year successor Zocor. Strategies to unearth blockbusters today are "not working," says Alberts, who retired in 1995 after 20 years at Merck. "I think that's fairly clear."

Graced by luck

What distinguishes past generations of drugmakers from the present? One difference is their starting point. Scientists then were often running blind, chasing new therapies without the benefits of modern biochemistry and the clues it can provide. Serendipity often planted the seeds of a new drug. Celebrex, Pfizer's antiarthritis drug, was chosen for further testing over another chemical in what was "more

CREDIT: CMSP



Protracted search. Ludo Kennis, shown working at Janssen Pharmaceuticals in the 1970s, chased several false leads before he hit on the antipsychotic Risperdal.

CREDIT: COURTESY OF LUDO KENNIS

or less a coin flip," says John Talley, one of its inventors. Talley did the work while at Searle, which later became part of Pfizer. He now heads drug discovery at Microbia, a biotech in Cambridge, Massachusetts.

But luck was only part of the equation. "Chance favors the prepared mind—is that how the saying goes?" asks Welch, who joined Pfizer's Groton, Connecticut, lab in 1970 and retired 3 years ago. Koe came to Pfizer in 1955 and studied penicillin offshoots before he was transferred to the company's tiny team of central nervous system researchers. After dabbling in potential antianxiety compounds, Koe turned to a then-new concept: the effects of serotonin in depression. He soon roped Welch into working on candidate antidepressants. Within a year he and Koe had hit on Zoloft.

One thing that hasn't changed for drug inventors, says Welch, is the need to stay abreast of developments in a given field. This is particularly crucial because, like drugmakers today, researchers are frequently transferred from their area of expertise into a new therapeutic area—say, from endocrinology to cardiology—and are expected to bring themselves up to speed quickly. Inventors recall their obsessive tracking of published chemical structures, patent filings by rival companies, and clinical trials of comparable drugs—even those that fail—as signposts helping point the way to their blockbuster.

Tenacity also helps. Without it, medicinal chemist Ludo Kennis would never have succeeded in creating the antipsychotic drug Risperdal, which grossed $3 billion last year for its parent Johnson & Johnson.

The late Paul Janssen, a pharmacologist who founded the company in 1953, wanted an alternative to the antipsychotic Haldol, whose patent was expiring. But competition in the field was fierce. Kennis, who joined Janssen's research unit near Antwerp, Belgium, in the mid-1960s, and his colleagues began with little more than a hunch that blocking the neurotransmitters dopamine and serotonin simultaneously might do the trick. But their knowledge of the underlying neuroscience was thin. It was a lucky goal set "without really knowing that this was a real improvement," says Kennis.

Every drug they made was tested in rats to see if it blunted dopamine and serotonin. Three compounds that did so progressed to human trials. "At that time, it was very easy to do a clinical experiment," says Kennis. Regulations governing human experimentation were far more lax than today's, he notes.

All three drugs failed. The most promising displayed wildly different metabolic properties in humans from those in rodents. But Kennis took another look at the compound, setoperone, and refashioned it. More than 20 years after he started, the U.S. Food and Drug Administration approved Risperdal.

Such delays were not uncommon. Merck's multibillion-dollar asthma and allergy drug Singulair entered clinical trials in 1994 after 13 years of sometimes tortuous development, including clinical trials of two related compounds that failed due to animal and human liver complications. There was a "willingness of the corporation to take these risks," says Robert Zamboni, one of Singulair's discoverers and now the vice president of medicinal chemistry at the company's Merck Frosst research center just outside Montreal in Canada. "These days, it wouldn't happen."

Kennis, now partly retired from Johnson & Johnson, says that nowadays "management people give us a certain period of time to find a new compound. … You cannot spend 10 years on something [for] which you don't know the outcome."

Facing doubters

The current skittishness about a drug's prospects might have sunk today's top-selling class of drugs, the statins. "There was no consensus then, as there is today, that lowering cholesterol [is] good," says Jonathan Tobert, who oversaw statin trials of Mevacor and Zocor at Merck and is now a consultant. Pursuing anticholesterol drugs, he says, "was a high-risk proposition."

In the 1960s, an anticholesterol drug from Marion Merrill Dow was withdrawn from the market after it was found to cause cataracts and other problems. Despite this troubled history, Merck stuck with a cholesterol program it had begun around that time, and Alberts was assigned to it after joining the company in 1975 as an expert on lipid synthesis. His chief competitor was Japan's Akira Endo, who worked at Sankyo; Endo had made breakthrough discoveries in cholesterol metabolism.

Alberts had little idea where to find anticholesterol compounds. Based in part on what he knew of Sankyo's work, he turned to extracts from fungus, which had been rejected by a Merck lab in Spain seeking new antimicrobials and contained a jumble of products made by the organism. In the first week, 17 samples arrived in the lab and failed to blunt a preselected cholesterol target; the 18th, which reached Alberts's group a week later, worked so well that Julie Chen, running tests alongside Alberts, was convinced she'd erred. She hadn't. The extract that became Mevacor surfaced after just 2 weeks in 1978.

That was the easy part. Despite the company's research in the area, many at Merck were leery of cholesterol drugs. A Merck medication had recently failed in clinical trials, says Alberts, and people from that project "had become very negative."

But back then, "a few people who really felt strongly about something could have quite an impact" on company decisions, says Tobert. And so, despite differences of opinion over its fate, the compound moved forward. It suffered a near-fatal setback in the fall of 1980, when a Sankyo cholesterol trial was abruptly halted due to safety issues in animals. Sankyo was quiet about what those were, but Merck's compound was similar enough to Sankyo's that its trials, too, stopped. Three years of analysis on Mevacor, and much "agitating" by Tobert, Alberts, and others, sent the drug back to the clinic. After a cataract scare in dogs, it was approved in 1987.

Mevacor and its successor Zocor, a slightly modified version of its parent in which Alberts played a lesser role, have brought in tens of billions of dollars for Merck. But Tobert thinks that Mevacor might have been "shot down" had it emerged more recently. In the 1980s, he says, "somebody [who felt] strongly about something" and was "nowhere near a vice president

… [was] heard." The company was also willing, if sometimes reluctantly, to buck traditional wisdom. Part of the change, Tobert thinks, is a result of steadily growing research and corporate staffs and the accompanying bureaucracy.

When the statin Lipitor was discovered in the mid-1980s, its backers faced a different challenge. Lipitor would be the fifth statin on the market, and in animals "it looked a little better [than existing statins], but not a lot, not enough to make a couple-million-dollar bet on," says Pfizer's Roth, then at Parke-Davis. Parke-Davis's marketing department predicted that Lipitor would earn no more than $350 million a year.

The company's chair of atherosclerosis and its head of research thought the drug deserved a chance, however, and coaxed senior management into funding a short-term clinical trial of healthy employees. The drug appeared to be more effective than any other statin. Today, by a $6 billion margin, it's the world's best-selling drug—and Roth, now a vice president of chemistry, swallows his own invention daily.

Reflecting on how drug discovery has changed since the days of Lipitor, Roth mourns the luxury of time that's been lost. But he also sees important enhancements. Regulatory hurdles mean that drugs developed today are safer, more selective, and more potent, he believes. Back then, "we didn't understand the science"—evinced by Parke-Davis's surprise at how well Lipitor performed in people. A drug's biology is still often baffling, but its behavior in humans is better understood, scientists agree.

Tobert, however, perceives a more fundamental breakdown in the drug discovery process. "A company's got to be prepared to take some risks," he says, risks that must be balanced against possible harm to patients. Public fears about drug safety are widespread these days, driven in part by recent revelations that blockbusters are not free of serious hazards: best-selling selective serotonin reuptake inhibitors such as Zoloft can trigger suicidality, and COX-2 inhibitors such as Celebrex and Vioxx increase the risk of heart attacks.

No financial windfall

Even if their drug is later roiled in controversy—an experience that Celebrex inventor Talley says he's found deeply distressing—scientists take enormous pride in their creations. Nearly all those interviewed recalled being told, in their early days as industry scientists, that few drug hunters ever find one that makes it to market. "Virtually everything we do fails," says Roth. Success, if it comes, tastes sweet.

But it doesn't fatten the discoverer's wallet. At best, scientists receive some stock options as a reward for their work. Although companies can informally thank researchers, such as with pay raises or promotions or internal awards, they do not normally offer drug discoverers a revenue share or a substantial cash reward once the drug reaches the market.

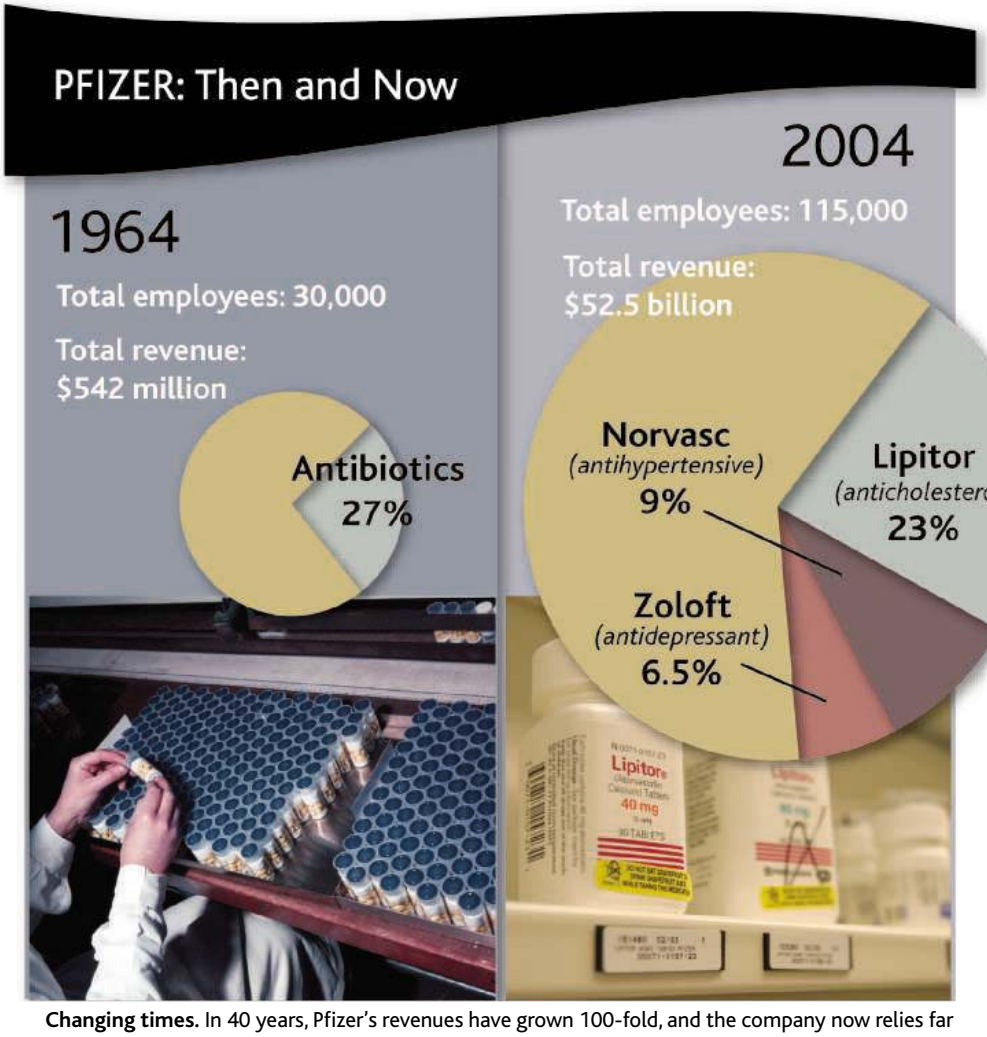
It's a policy many would like to see changed. Talley, who in addition to Celebrex discovered the related drug Bextra (which was recently removed from the U.S. and European markets due to safety concerns), struggled to pay college tuition for his two daughters while the therapies he created brought in billions for the company. "The ideal … would be to get royalties," says Alberts, who discovered Mevacor. "I don't know how to do it, but I think there should be a better way."

Not everyone agrees. Roth and some others say the current setup—in which companies suffer the risks and reap the benefits, and scientists enjoy a steady salary whether they hit on a blockbuster or not—may be the fairest. Monetary rewards could "create an enormous competition [between] people internally," says Kennis, a competition he believes would be unhealthy. Pharmaceutical giants such as Pfizer, AstraZeneca, and GlaxoSmithKline say they do not pay discoverers of new drugs for their finds. It's not something his pharmaceutical industry group has been asked to consider, says Jeffrey Trewhitt, spokesperson for the Pharmaceutical Research and Manufacturers of America in Washington, D.C.

In the end, it's not lack of financial reward that drives the occasional inventor to leave the place where his discovery was made. Rather, what most bothers these scientists is how drug discovery has evolved. Company management increasingly favors "the short-term developmental route" rather than investing in projects more speculative in nature, says Craig Smith, the co-inventor, with Raymond Goodwin, of the rheumatoid arthritis drug Enbrel. The pair discovered Enbrel while at the Seattle, Washington, biotech Immunex, initially working on the side when their bosses "weren't looking," says Smith. Both left after Immunex was bought for $16 billion by biotech giant Amgen in 2001.

Smith predicts that drugmakers who avoid untrodden territory, based on inquiries that may not lead anywhere, will hit a wall down the road. "In the longer run," he says, "you're shooting yourself in the foot."

—JENNIFER COUZIN



Changing times. In 40 years, Pfizer's revenues have grown 100-fold, and the company now relies far more heavily on the popularity of individual drugs—in particular, its $12-billion-a-year Lipitor.

SOURCE: PFIZER; IMAGES: CORBIS

NEWS

Saving the Mind Faces High Hurdles

Fierce competition to find a drug that could delay onset of or prevent Alzheimer's disease is a relatively recent phenomenon. Why was this potential blockbuster shunned for so long?

Cancer has been arguably the most feared disease in the United States for the past several decades. Now, as the baby boom generation starts to inch past middle age, a new contender has emerged for that unappealing label: Alzheimer's disease (AD).

An estimated 4.5 million Americans already have the neurodegenerative condition, and that number could more than triple by 2050. Devastating to both those afflicted and their caregivers, the illness exerts a $100-billion-a-year drain on the U.S. economy, according to the Biotechnology Industry Organization. "Alzheimer's disease probably has a larger impact on society than any other disease, in terms of economic and emotional costs," says Dale Schenk, chief scientific officer at Elan, a biotechnology company based in Dublin, Ireland.

So when *Science* looked for a condition that illustrates the challenges confronting the pharmaceutical industry—and the opportunities that beckon—AD was an obvious candidate. A drug that slows the disease could be especially lucrative because it presumably would need to be taken well before the first symptoms are likely to appear, and then for life. "Everyone recognizes that this is a great, unmet medical need. The drug company that succeeds here will be a *very* successful company," says Peter Boxer, associate director of central nervous system (CNS) pharmacology at Pfizer Global Research and Development in Ann Arbor, Michigan.

That recognition is fairly new, however. Academic and federal scientists had to lobby hard in the late 1980s to get Parke-Davis to conduct the first major clinical trial of an Alzheimer's drug. Although that drug, tacrine, and related compounds known as acetycholinesterase inhibitors rang up about $3 billion in sales for AD therapy in 2003, they are less than ideal medicines. They don't halt the underlying progression of the disease, and their slowing of cognitive decline is temporary.

But even a less-than-perfect AD drug could still be a blockbuster for companies.

Close look. By studying brain tissue from people who had Alzheimer's disease, drug companies are racing to develop drugs that slow or treat the disorder.

CREDIT: SIMON FRASER/NEWCASTLE GENERAL HOSPITAL/PHOTO RESEARCHERS INC.

It would also be a boon for society: Because the prevalence of Alzheimer's disease increases exponentially with age, drugs that provide a modest 5-year delay in the onset of symptoms would reduce the number of affected people by as much as 50%.

> "Everyone recognizes this is a great, unmet medical need. The drug company that succeeds here will be a very successful company."
> —PETER BOXER, PFIZER

Industrial nihilism

Although drug development for AD is a relatively young endeavor, the condition was identified nearly a century ago by German neuropathologist and psychiatrist Alois Alzheimer. In 1906, he gave a lecture on a 51-year-old woman who had died with dementia. An autopsy found that her brain was littered with extracellular masses (plaques) and intracellular clumps (neurofibrillary tangles) that have since become the diagnostic hallmark of the disease that now bears his name. But for decades, because it wasn't diagnosable until after death, AD remained an obscure condition, and study of the illness was a scientific backwater. "No one wanted to get into [AD research] because it was seen as an unpromising career path leading to a scientific dead end," recalls Zaven Khachaturian, former director of the Office of Alzheimer's Disease Research at the National Institute on Aging (NIA).

The same pessimism about AD held true in industry. "There was very little interest because the disease could not be diagnosed, and the prevailing wisdom considered it an untreatable normal consequence of aging," says Khachaturian. The lack of a cause was equally stifling to drug development. "There was a nihilism around [AD]," says neuroscientist Geoff Dunbar, who has worked on CNS drugs at several major companies and is now at a small biotech firm, Targacept, in Winston-Salem, North Carolina. "No one knew what to do with the plaques and tangles."

In the absence of hard evidence, a few vague theories took root. Some researchers argued that the dementia in general stemmed from inadequate blood flow within the brain, giving a slight boost to a class of drugs called cerebral vasodilators. Similarly, compounds that promoted learning and memory in animals—drugs known as nootropics, which means "growing the mind"—were also suggested as dementia treatments. "The assumption was that that would be sufficient to help the deficits in Alzheimer's disease," recalls Boxer.

The scientific hook

Drug development for AD didn't truly get started until the cholinergic hypothesis emerged in the late 1970s, largely through the efforts of British neuroscientists such as Peter Davies, now at Albert Einstein College of Medicine in New York City. In 1976, for example, he and a colleague reported that compared to normal brains, those from several people who had had the brain disorder had decreased levels of an enzyme that helps make the neuro-

transmitter acetylcholine. Those data, combined with earlier evidence that drugs blocking the cholinergic system produced memory problems in people, led Davies and others to argue that the core defect in AD was a lack of acetylcholine.

"Until that time, dementia was primarily looked at as an amorphous mental disorder," says Khachaturian. "The cholinergic hypothesis was the first scientific hook that could provide a clear path to understand the underlying neurochemistry of AD. It also gave us a plausible scientific rationale for developing treatments because so much was known about the cholinergic system." That knowledge, says Dunbar, "meant we were in neuropharmacology that the industry understood."

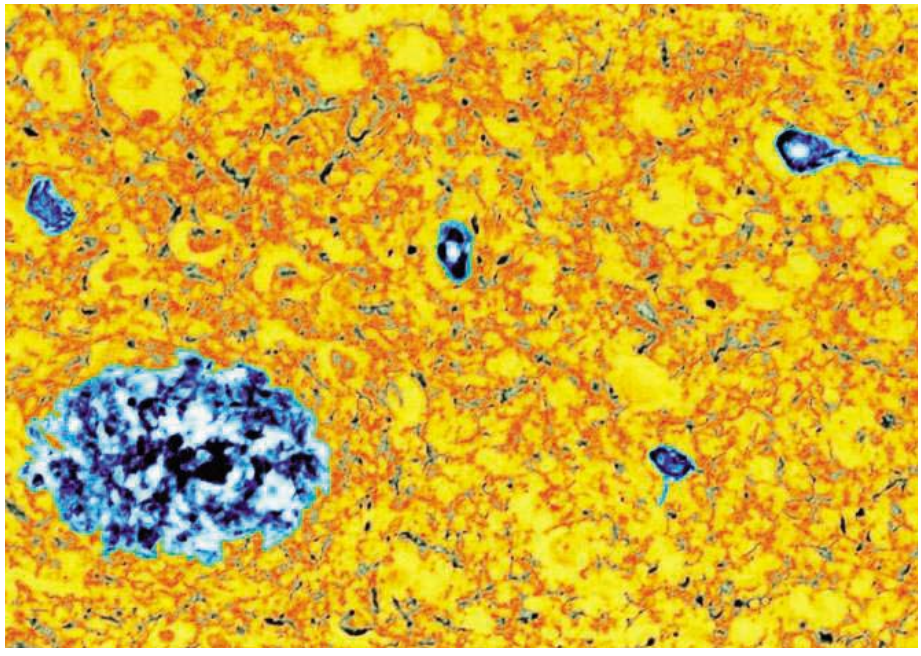
There was also an obvious therapeutic road map to follow. It drew from work a decade earlier showing that the symptoms of Parkinson's disease stemmed from the death of dopamine-producing neurons and that L-dopa, a dopamine precursor, could bring about miraculous recoveries in patients. Could curing AD, researchers asked, be as simple as replacing acetylcholine?

Not quite. Efforts to deliver acetylcholine precursors to the brain met with little success. In 1986, however, a different strategy grabbed the spotlight. A research team reported remarkable benefits for a few AD patients taking the well-studied compound oral tetrahydroaminoacridine, also called tacrine, which blocks the activity of an enzyme that breaks down acetylcholine.

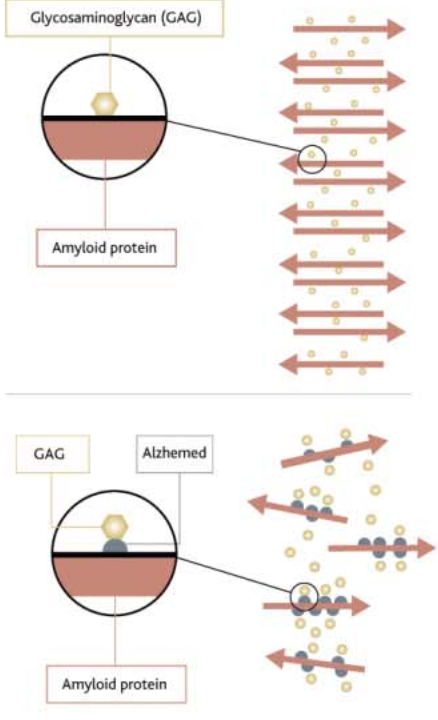
Quickly deciding to push for a validation study on the efficacy of tacrine, Khachaturian and the directors of the recently created, NIA-funded network of Alzheimer's Disease Research Centers sought a company to formulate the compound, which was off-patent, into various doses and quantities needed for a full-scale trial. They found an advocate in Elkan Gamzu at Parke-Davis. "Having a person inside that company lobbying for an efficacy study was very important to getting that first drug to go," says Khachaturian.

Parke-Davis, a division of Warner-Lambert Co. that later became part of Pfizer, started its tacrine study in 1987. But the drug, marketed as Cognex, failed to pass muster with a Food and Drug Administration (FDA) advisory board in 1991. After further trials with higher doses, the drug won FDA approval in 1993, albeit not without controversy. "The scientific community was not very enthusiastic about it because the benefits were marginal and it had a lot of side effects," says Khachaturian.

Still, its approval validated the cognitive tests that had recently been developed to gauge drug efficacy for the disease and provided clear guidelines on how to conduct clinical trials for AD. "If the FDA had set the bar very high and not approved it, then that would have been the kiss of death. No other company would have gotten into developing [AD] therapies," says Khachaturian. "Once tacrine was approved, a lot of other companies jumped on the bandwagon" to develop safer and more potent acetylcholinesterase inhibitors, notes Boxer. (Six of the seven drugs currently approved in the U.S. for AD are in this class.)



Double trouble. The pathological hallmarks of Alzheimer's disease are extracellular brain deposits of β amyloid called plaques (large blue oval in corner) and intracellular clumps of tau known as tangles (smaller blue masses).



Keep apart? Now in phase III study, the drug Alzhemed blocks proteoglycan molecules from helping β amyloid form fibrils.

The quick follow-up to tacrine by other drugs targeting the same enzyme illustrates an important principle of drug development. Even before a company with a head start on a target proves the value of a class of drugs, other firms will generally have similarly acting "me, too" drugs with improved properties in their pipeline. For competition's sake, says Boxer, "you can't wait for other companies' clinical data."

The acetylcholinesterase inhibitors spurred research into other ways of tweaking the cholinergic system. Acetylcholine operates through two classes of receptors, muscarinic and nicotinic, and major pharmaceutical companies vigorously pursued muscarinic agonists until troublesome side effects slowed their development. "Big pharma is still plugging away at the muscarinic hypothesis," says Dunbar. That has left room for his current firm, Targacept, to develop AD drugs that target nicotinic receptors.

The amyloid hypothesis

Still, halting the decline of the cholinergic system in AD is not the same as curing, preventing, or even slowing the actual pathology of the illness. In fact, the benefits of acetylcholinesterase inhibitors are so questionable that a government panel evaluating drugs for the U.K. health care system recently issued a preliminary opinion that the drugs aren't worth buying, a viewpoint the makers of the drugs have strongly challenged.

CREDITS (TOP TO BOTTOM): SIMON FRASER/PHOTO RESEARCHERS INC.; ADAPTED FROM THOMAS JEFFERSON UNIVERSITY

Most companies seeking more fundamental treatments for AD are focusing on a protein fragment called β amyloid, which in 1984 was shown to be the primary component of the brain's plaques. That discovery spawned the amyloid hypothesis, which holds that the buildup of β amyloid causes AD by harming or killing brain cells. In 1991, scientists found that several families plagued by an early-onset form of AD had mutations in the gene encoding β amyloid precursor protein (APP), from which β amyloid is derived. A few years later, similar disease-causing mutations were found in genes encoding proteins called presenilins that were subsequently shown to affect APP processing into β amyloid.

The amyloid hypothesis provided a bounty of new targets and potential strategies. Some companies tried to prevent β-amyloid molecules from clumping together, for example, while others began testing whether known drugs, such as statins and nonsteroidal anti-inflammatories, alter β-amyloid production.

The novel hypothesis opened the door for small biotech companies, too. Neurochem, which was founded in 1993 in Laval, Canada, drew upon research licensed from Queen's University in Kingston regarding proteoglycan molecules in the brain that bind to β amyloid and promote the formation of the amyloid fibrils that make up plaques. The company has developed small organic molecules that mimic these proteoglycans, occupying their binding sites on β amyloid and preventing fibrils. Earlier this year, Neurochem launched a phase III trial of its lead Alzheimer's treatment, Alzhemed, seeking to become the first to bring an amyloid-modifying drug to market.

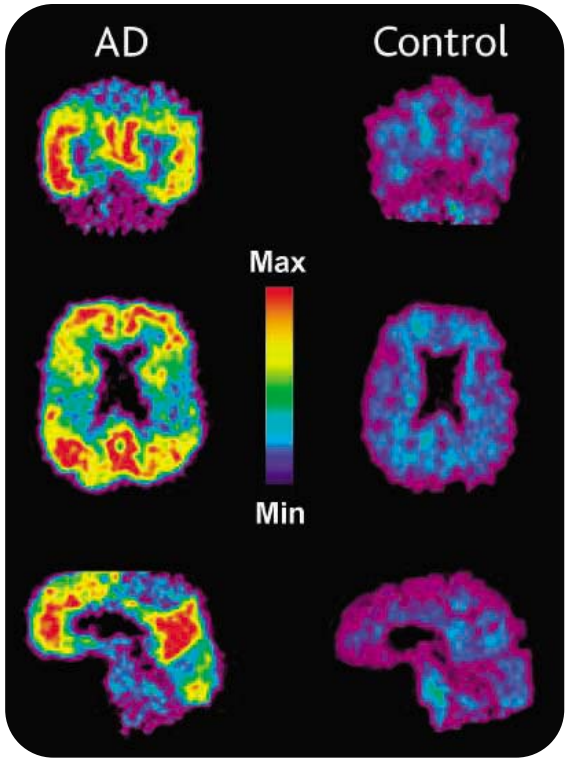
Few firms are trying to directly block β-amyloid molecules from aggregating, notes Dennis Garceau, senior vice president of drug development at Neurochem. "Big companies like to target enzymes; it's a more conventional target," he says. Indeed, the fiercest competition has been to develop secretase inhibitors, compounds that block the enzymes that cut APP into the smaller β-amyloid fragment.

The race began in 1999 when a β secretase that acts upon APP was identified. (After the initial published report by Amgen, several other firms quickly revealed that they too had identified the same potential β secretase, perhaps setting the stage for a patent fight.) "Everyone went after that target right away. It was such a rational target," says Boxer, who recalls hearing that another company had launched a major effort to inhibit the enzyme within a week of the announcement of its discovery.

The identified β secretase was a particularly inviting target because it belonged to the same family of enzymes as HIV's protease. Several protease inhibitors had already been approved as AIDS drugs, allowing companies to draw on those experiences.

It takes two cuts to make β amyloid out of APP, however. Drug companies weren't ignoring the other key enzyme, γ secretase, but they just weren't clear what it was. A theory that presenilins were γ secretases took several years to be accepted after its 1999 proposal. Still, even without a clear identification of the enzyme, several firms had developed in vitro systems displaying γ-secretase activity upon which they could test potential inhibitors.

Current efforts to develop secretase inhibitors remain shrouded in corporate secrecy. Bristol-Myers Squibb reportedly began clinical testing a γ-secretase inhibitor in 2001 and stopped because of side effects, but it has never publicly reported those results. Eli Lilly has also just begun clinical testing of a γ-secretase inhibitor. The challenge in developing such drugs seems to be blocking their action on enzymes needed for activities other than cutting up APP. γ secretases also cleave a protein called Notch, for example, that's important in development and the immune system. As a result, companies must find compounds that more specifically affect APP processing.



PET project. Using a compound developed at the University of Pittsburgh, researchers can now use PET scans to image the amount of β amyloid in brains of people with (*left*) or without (*right*) Alzheimer's disease. Such an ability could help drug companies monitor whether a drug is helping a person with Alzheimer's disease.

CREDIT: UNIVERSITY OF PITTSBURGH

A surprise vaccine

While the amyloid hypothesis has offered drug researchers a number of obvious targets and strategies, it also led to the most surprising attempt to thwart AD. In the late 1990s, long after his colleagues at Elan had tested their most promising compounds, Schenk suggested injecting a few mice with β amyloid itself. His goal was to raise an antibody or other immune response against plaques. "No one thought it would work. Even after the experiment was done, the results weren't analyzed for a while," recalls Schenk.

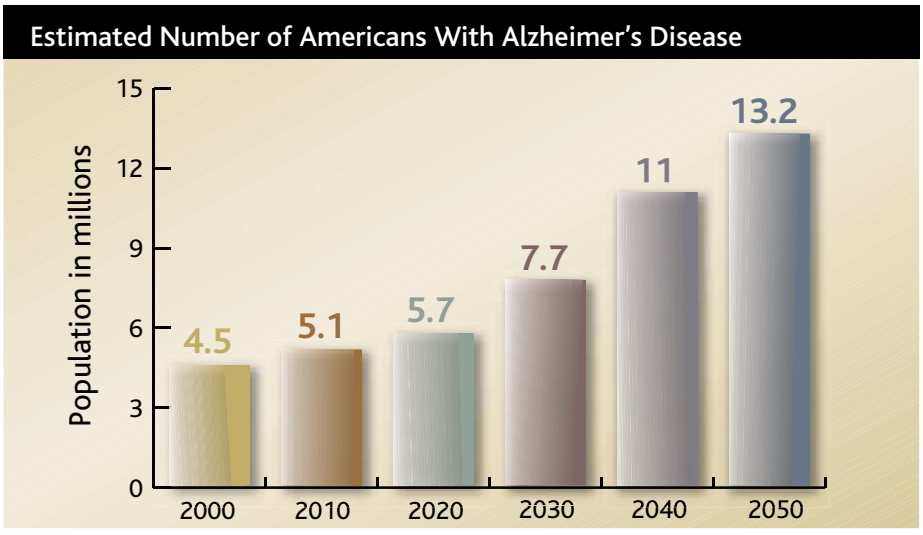
The results were stunning. The immunization slowed or prevented the development of β-amyloid plaques in young mice and even wiped away preexisting ones in older mice. The episode illustrates how one person's idea can change the direction of a company or a field. "Dale was really brave," says John Trojanowski of the University of Pennsylvania School of Medicine in Philadelphia.

How does big pharma react when a disease-treating strategy such as the Elan vaccine comes out of the blue? Most large companies working on CNS drugs have experience with small-molecule drugs, not antibodies, says Boxer. And although firms can always tweak an enzyme inhibitor to make a better drug and carve out some market share, vaccines tend to either work or not. "We look at this stuff and go, 'Huh?' " says Boxer. "Where is your unique drug?" As result, he says, most companies have conceded the vaccine approach to Elan.

The unexpected emergence of the Elan vaccine illustrates the importance and limitations of animal models. For several years, companies pursuing the amyloid hypothesis were largely stuck in vitro. Attempts to genetically engineer mice that overproduced APP seemed fruitless; there was even a notable fraud case in which a researcher published a picture of a human plaque as evidence that his mice had developed β-amyloid clumps. "The entire field was trying to make a mouse model," says Schenk.

Then a failing biotech company trying to sell off its assets approached Elan and saved the day, ultimately setting the stage for the vaccine's proof of principle. The struggling company's transgenic rodents were greatly overexpressing APP, and when Elan scientists checked out the mice, they found numerous brain plaques. Elan acquired the rights to the mice and quickly began testing its compounds. The company eventually allowed the β-amyloid vaccine strategy to be tested. Without that animal model, the idea might have faded away.

Having animal models reduces the risk, and thus the cost, of developing drugs. For small



Going up. A number of factors influence predictions of how many Americans will have Alzheimer's disease in the coming years, but all such estimates suggest a rapid increase.

companies such as Neurochem, they can also be a lifeline to continued funding from venture capitalists and other sources. "Until we got proof of concept in vivo, people were a little bit skeptical," says Garceau.

Yet animal models also reveal the risks of drug development. Elan's vaccine approach seemed to work well in mice, but brain inflammation in a few patients triggered an abrupt halt to the clinical trial. Elan, together with its partner Wyeth, is now conducting clinical trials with plaque-targeting immunotherapy strategies such as passive administration of antibodies to β amyloid.

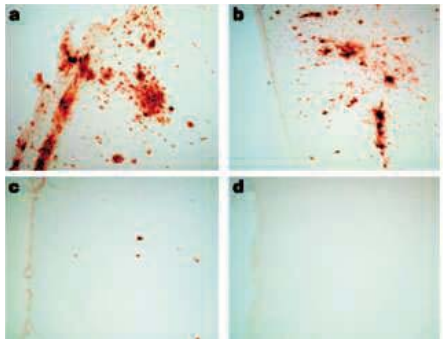
But how can a company pursuing β amyloid–based therapies for AD know if its drug or treatment is working? Showing that people maintain the same cognitive and memory skills, or improve such skills, can be difficult and time-consuming. Unfortunately, there are no well-accepted AD biomarkers, like cholesterol levels for heart disease or viral load for AIDS. A lack of animal models and biomarkers are "two difficult issues for developing a drug," says Boxer.

The biomarker obstacle has led companies such as Pfizer, Merck, Eli Lilly, and Elan to partner with the Alzheimer's Association, NIA, the National Institute of Biomedical Imaging and Bioengineering, and FDA to identify ways of measuring progression of mild cognitive impairment and AD in people. Industry will pick up one-third of the cost of the $60 million, 5-year effort, known as the Alzheimer's Disease Neuroimaging Initiative, that will test various ways of imaging brain plaques and tangles as well as measuring levels of proteins in blood, urine, and cerebrospinal fluid. "It's so difficult [to develop an Alzheimer's treatment without biomarkers] that the drug companies are collaborating," says Boxer.

What about tau?

It's sometimes forgotten that the effort to develop β amyloid–based treatments represents a huge and costly gamble on a single, unverified theory of AD. There are many other hypotheses being explored by small numbers of scientists or a handful of tiny biotech firms. One is the second major theory of AD, which involves tangles, the intracellular brain lesions identified by Alois Alzheimer.

In the early days, Alzheimer's researchers were divided over whether plaques or tangles were more important. The identification of β amyloid in plaques and disease-causing mutations in the *APP* gene relegated tangles and their primary constituent, a hyperphosphorylated form of a protein called tau, to a sideshow. "We were the token other pathway at every meeting," recalls Trojanowski; he and his wife Virginia Lee have been the most vocal proponents of tangles and tau research.



Surprise shot. Mice genetically engineered to overproduce β amyloid develop brain deposits (a, b) similar to the plaques in Alzheimer's disease, but injecting such rodents with β amyloid stirs an immune response that can clear such deposits (c, d).

For companies, that lack of interest was partly a matter of simple economics. "Even big pharma can only pick a certain number of targets," says Dunbar, noting that Bristol-Myers Squibb, where he used to direct clinical development of CNS drugs, has never had a tau program to his knowledge.

Tau is now drawing more attention, in part because of a 1998 paper in which researchers showed that mutations in a gene encoding one of the human versions of tau lead to a rare form of dementia that bears some similarities, such as tau tangles, to AD. "It launched studies that should have been done in the early 1990s," says Trojanowski.

Trojanowski contends that tau, when it becomes overloaded with phosphate groups, can no longer bind to and stabilize cellular filaments called microtubules. That change disrupts the ability of neurons to transport molecules down the long extensions known as axons. Back in 1994, his team proposed that microtubule-stabilizing compounds, such as the cancer drug Taxol, might treat AD. And earlier this year, in the 4 January *Proceedings of the National Academy of Sciences*, they offered a proof of concept in mice genetically engineered to overproduce a human version of tau.

These rodents suffer from a neurodegenerative disorder that includes tanglelike masses of hyperphosphorylated tau and impaired axon function. As hypothesized, the administration of Taxol sped up the animals' axonal transport and ameliorated their motor problems. Trojanowksi and his colleagues are now working with Angiotech Pharmaceuticals in Vancouver, British Columbia, and other firms are sniffing around. "I know pharma is interested," he says. "My phone rings more often."

Partnerships and future

Will the next significant drug for AD come from a small biotech company or big pharma? Given the economics of drug development, it's likely that the Davids and Goliaths will end up working together.

"It's very difficult for a small company to take a drug all the way to market," notes Targacept's Dunbar. His company's strategy, for example, is to push a drug only through phase II trials and then "outsource it to big pharma." And Neurochem says it would be open to partnerships with bigger companies given the right deal.

Big pharma is certainly happy to let smaller companies take the initial plunge before it swoops in and buys up a promising drug. "They have such big wallets they can wait until almost all the risk is taken out," says Dunbar.

Still, the search for Alzheimer's drugs should leave room for many companies, small and large, to prosper. "This disease will need a cocktail of treatments," predicts Neurochem's Garceau. **–JOHN TRAVIS**

CREDITS (TOP TO BOTTOM): HERBERT *ET AL. ARCH. NEUROL.* **60**, 1119–1122 (2003); *NATURE* **400**, 173–177 (1999)

NEWS

Pharma Moves Ahead Cautiously in China

Companies can't resist the lure of China. But full-service research labs remain on the horizon

SHANGHAI—As recently as 5 years ago, China was terra incognito for big pharma research organizations. To be sure, the global drug giants have been selling their products in China since the 1980s, and quite a few have built manufacturing plants there. Yet concerns over enforcement of the country's fledgling laws governing intellectual property rights (IPR) had prevented companies from taking the logical next step: opening a lab to do drug discovery.

Those qualms remain. But they are balanced by the industry's growing desire to dip into China's intellectual talent pool. In 2002, Novo Nordisk broke from the pack and set up a small research facility in Beijing, the company's only research site outside its home in Denmark. Later that year, U.K.-based AstraZeneca set up the first Western-owned clinical research organization in China to collaborate on multisite trials. The next year, U.S.-based Eli Lilly inked a deal with the Chinese company ChemExplorer to purify, synthesize, and analyze compounds supplied by its researchers. And last fall, when the Swiss-based Roche dedicated its new research and development lab in Shanghai, Roche Chair and CEO Franz Humer predicted that China will "someday [be] one of Roche's important R&D centers rather than a mere market and production base."

Humer may well be right. And yet, his open-ended time reference sent the subtle but unmistakable message that big pharma still harbors doubts about China's ability to protect any valuable intellectual property that a company might create within its borders. Indeed, the research director of the Novo Nordisk site, Wang Baoping, concedes that his company is taking a risk. "China has in place a series of laws related to IPR protection. But their enforcement, particularly the amount that would be paid to the damaged side, remains a problem that needs to be addressed," says Wang, a U.S.-trained geneticist. "I am not sure when the IPR environment in China will be truly favorable."

China's growing appetite for Western drugs—the current $15 billion market is expected to quadruple by 2010, and then double again by 2020—has certainly caught the attention of every drug company. So has its cheap but skilled scientific labor force. Not only do Ph.D.s receive annual salaries of $10,000 or less, but the most expensive aspect of drug development—clinical trials—costs an estimated 30% less in China than in the United States or Europe. And then there is its growing prowess in science. "I'd say that setting up our own research lab there is only a matter of time," Novartis CEO Dan Vasella remarked this spring. "It's not so much a need as it is a hunger to take advantage of the opportunities."

Despite those inducements, the research centers being set up are shadows of pharma's existing full-service shops in the West. The Novo Nordisk and Roche labs are much smaller—some 40 to 50 scientists—and narrower in focus, typically medicinal chemists and biologists. But company officials still hope that the labs can make big contributions. Roche's Chen Li, chief scientific officer for the Shanghai site, says the key to its success in medicinal chemistry will be "giving full play to the initiatives of the scientists here, including access to information" throughout Roche's global research network. At Novo Nordisk's Beijing lab, scientists focus on protein expression to supplement the company's portfolio of diabetes drugs.

Lorenz K. Ng, vice president of research alliance and business development for Lilly Asia, says, "We looked at China because of its good supply of chemists." Its partner ChemExplorer has a team of 175 chemists. Still, as one ChemExplorer scientist notes, "What we do is only one piece of the core technology of drug development." AstraZeneca's clinical research unit focuses on another piece of drug development by taking advantage of lower costs and access to a different population. The unit has been involved in six multicenter trials, totaling 765 patients. And Pfizer China is recruiting biometricians to staff a clinical trials data management center that it hopes to open early next year to help the company crunch the numbers from trials already under way.

Most Chinese scientists believe the arrival of Western pharmaceutical companies will be a long-term benefit for the country. "The biggest contribution of foreign research centers is the opportunity to learn drug development. That's a huge gap that needs to be filled in China," says Hu Zhuohan, a professor of pharmacology at Fudan University in Shanghai.

But a few observers worry that the trend will stifle China's own efforts. "Research and development is one of the least profitable links of the pharmaceutical chain," says Zhang Hua, a financial analyst in east China's Shandong Province, who predicts that small, young local drug companies "will inevitably fall prey to foreign companies." Even so, Zhang thinks the process is irreversible, and he holds out hope that Chinese companies will learn drug innovation more quickly by watching it firsthand.

—GONG YIDONG

Gong Yidong writes for *China Features* in Beijing.

First steps. Chinese scientists at work in the Novo Nordisk R&D Center in Beijing.

CREDIT: NOVO NORDISK

Courting Bird Sings with Stridulating Wing Feathers

Kimberly S. Bostwick[1]* and Richard O. Prum[2]

Since Darwin's time (*1*), the nonvocal, feather-produced sounds of birds have been hypothesized to have evolved by sexual selection (*2*). Here we describe an acoustic signal that is produced by a mechanism unique among vertebrates. High-speed digital video recordings of the courtship displays of male club-winged manakins, *Machaeropterus deliciosus,* show that males produce sustained harmonic tones through interactions among oscillating secondary wing feathers.

The Neotropical manakins (Pipridae) are polygynous, lek-breeding birds in which sonation has evolved multiple times (*2*, *3*). The courtship sonation produced by male *M. deliciosus* sounds like a ringing *Tick–Tick–Ting* (audio S1) (*4*). The sound is as loud as a typical avian vocalization, easily audible from tens of meters away (*4*). The *Tick* notes are sharp tonal clicks, whereas the *Ting* is a sustained, violinlike note ~0.335 s long. Each sound is composed of fundamental frequencies of 1.59 and 1.49 kHz, respectively, with a series of harmonics that are integer multiples of the fundamental (Fig. 1A), a pattern characteristic of resonator-coupled stridulation (*5*, *6*).

The secondary, or inner, feathers have enlarged rachi, or shafts, that form clublike structures. The rachi of the sixth and seventh secondaries are greatly enlarged and hollow. They are twisted nearly 90° along their longitudinal axis so that, at their tips, their dorsal surfaces face medially and the feathers nestle closely together. In contrast, the distal ~12 mm of the rachis of the fifth secondary feather tapers abruptly to a thin, stiff, blade that bends medially to contact the ventral surfaces of the distal tips of the adjacent sixth and seventh secondaries (Fig. 1B). The surface of the sixth secondary has a set of 6 to 8 low, rounded ridges where the stiff tip of the fifth secondary contacts it (Fig. 1C).

Sonations are made while the male is perched (movies S1 and S2). To produce the *Tick* note, the wings are pronated (i.e., rotated forward), rapidly flipping the wing feathers above the bird's back (Fig. 1D). Simultaneously, the male adducts (pulls in medially) the wings, so that the tips of the modified secondary feathers strike together once forcefully across the back. The *Ting* note is produced using these same initial motions. However, after the initial contact of the secondaries over the back, the male shivers the wings, abducting (laterally extending) and adducting (pulling in) the forelimbs in a repeated cycle. This movement causes the flight feathers to oscillate lateromedially above the back (Fig. 1E). Each adduction culminates in a collision between the enlarged seventh secondary feathers above the back, which rebound after ~3 ms, initiating the abduction phase. After the abduction phase, the manus, or hand, is again adducted, redirecting the oscillating secondaries inward. The rate at which the feathers oscillate is 106.06 to 107.10 Hz (n = 3 Tings); thus, a frequency multiplier of 14 is needed to explain the fundamental frequency of 1.49 kHz.

We propose that the rapid pronation and adduction of the wings and the collision between the right and left secondaries produce an impulsive, mechanical excitation that induces the enlarged, hollow sixth and seventh feathers to resonate. The oscillations of the secondary feathers cause the tip of the fifth secondary to rub back and forth against the ribbed surface of the adjacent sixth secondary. The resulting mechanical input and the repeated collisions sustain the resonance of the sixth and seventh secondaries for the duration of the oscillations. The bent shape of the fifth secondary creates relative movement between the plectrum and file as the feathers oscillate, causing the plectrum to rub across the ribbed file twice (once medially and once laterally) for each wing cycle (Fig. 1F).

The derived morphological, mechanistic, and behavioral novelties of *M. deliciosus* are an extreme example of evolutionary modification of a locomotory structure for the production of an acoustic advertisement. The stridulation mechanism used by *M. deliciosus* to produce tonal sounds shows marked convergences with sound production in many insects, including the use of hardened integumentary appendages, extremely rapid limb vibration, and frequency multiplication through pick-and-file stridulation.

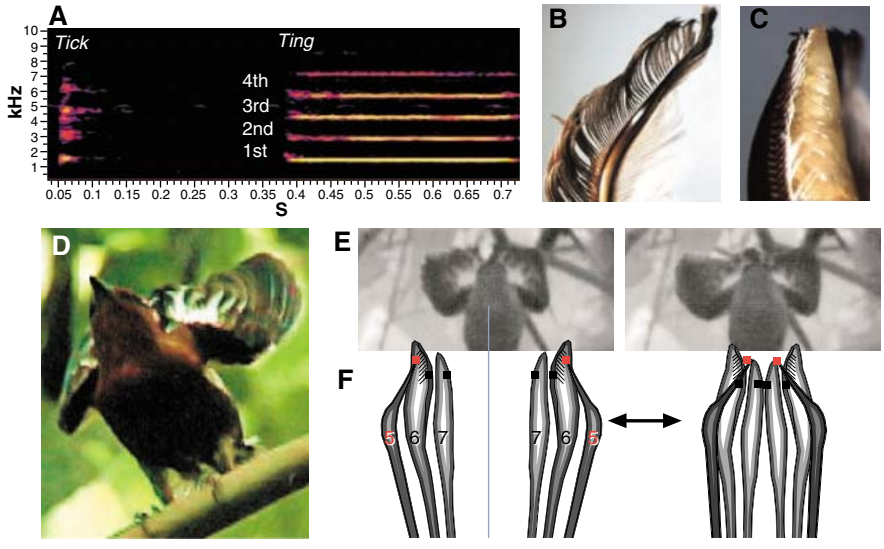


Fig. 1. (**A**) Spectrogram of a *Tick-Ting* sonation of one male *M. deliciosus*, showing signal energy in integer-related harmonic bands (labeled 1 to 4). S, seconds. (**B**) Dorsal surface of the right fifth secondary feather of a male *M. deliciosus*, the distal tip bent medially at 45°. (**C**) The medial surface of the thickened distal end of the right sixth secondary, showing regular, raised ridges. (**D**) Conventional and (**E**) high-speed video of *M. deliciosus* during *Ting* production. (**F**) Graphical depiction of the distal ends of the fifth secondary (the pick) moving across the ribbed surface of the sixth secondary (the file) to create stridulatory impulses that sustain the resonance of the enlarged sixth and likely seventh secondary shafts. Relative motion of the pick and file is indicated by red and black blocks, respectively; the gray line indicates the midline.

References and Notes

1. C. Darwin, *The Descent of Man and Selection in Relation to Sex* (John Murray, London, 1871).
2. R. O. Prum, *Anim. Behav.* **55**, 977 (1998).
3. K. S. Bostwick, R. O. Prum, *J. Exp. Biol.* **206**, 3693 (2003).
4. K. S. Bostwick, *Auk* **117**, 465 (2000).
5. N. H. Fletcher, *Acoustic Systems in Biology* (Oxford Univ. Press, New York, 1992).
6. H. C. Bennet-Clark, in *Cricket Behavior and Neurobiology*, F. Huber, T. E. Moore, W. Loher, Eds. (Cornell Univ. Press, Ithaca, NY, 1989), pp. 227–261.
7. Supported by NSF Doctoral Dissertation Improvement Grant no. DEB-0073289.

Supporting Online Material

www.sciencemag.org/cgi/content/full/309/5735/736/DC1
Materials and Methods
Audio S1
Movies S1 and S2

2 March 2005; accepted 28 April 2005
10.1126/science.1111701

[1]Department of Ecology and Evolutionary Biology, Cornell University, Ithaca, NY 14850, USA. [2]Department of Ecology and Evolutionary Biology and Peabody Museum of Natural History, Yale University, New Haven, CT 06520, USA.

*To whom correspondence should be addressed. E-mail: ksb6@cornell.edu

Supernova Olivine from Cometary Dust

Scott Messenger,[1]* Lindsay P. Keller,[1] Dante S. Lauretta[2]

An interplanetary dust particle contains a submicrometer crystalline silicate aggregate of probable supernova origin. The grain has a pronounced enrichment in $^{18}O/^{16}O$ (13 times the solar value) and depletions in $^{17}O/^{16}O$ (one-third solar) and $^{29}Si/^{28}Si$ (<0.8 times solar), indicative of formation from a type II supernova. The aggregate contains olivine (forsterite 83) grains <100 nanometers in size, with microstructures that are consistent with minimal thermal alteration. This unusually iron-rich olivine grain could have formed by equilibrium condensation from cooling supernova ejecta if several different nucleosynthetic zones mixed in the proper proportions. The supernova grain is also partially encased in nitrogen-15–rich organic matter that likely formed in a presolar cold molecular cloud.

Primitive meteorites and interplanetary dust particles (IDPs) contain small amounts of micrometer-sized presolar grains (stardust) that originated from evolved stars, novae, and supernovae. These grains provide direct probes of astrophysical environments that are inaccessible to traditional astronomical techniques (*1*). Stardust identified to date includes grains of diamond, Si_3N_4, SiC, graphite, TiC, Al_2O_3, TiO_2, hibonite, spinel, forsterite, and amorphous silicates (*2*, *3*). Grains of extrasolar origin are identified by isotopic compositions that differ from solar isotopic ratios, in some cases by orders of magnitude.

Silicates are an abundant form of dust in the galaxy, giving rise to strong infrared (IR) spectral features in outflows of evolved stars (*4*, *5*), the diffuse interstellar medium (ISM) (*6*), and disks around young stellar objects (*7*). Silicates are identified by their diagnostic 9.7 μm and 18 μm IR bands due to Si-O stretch and O-Si-O bending modes. The general lack of fine structure in these bands is usually attributed to the silicate dust being primarily amorphous in the diffuse ISM, although some far-IR spectra have revealed the presence of ~10% to 20% crystalline silicates around young and old stars. The scarcity of crystalline silicates in the diffuse ISM (~0.2%) thus suggests that they are destroyed or rendered amorphous by shock, sputtering, or collision within ~10 million years after they form in stars (*6*, *8*). The discovery of presolar silicates in meteorites and IDPs provides new insight into interstellar silicate mineralogy. To date, 6 of the 132 presolar silicates found thus far have been studied by transmission electron microscopy (TEM), including 2 forsterite grains and 4 amorphous silicates (*3*, *9*–*11*, this work).

Although silicates are the most abundant type of presolar grains, they were only discovered recently because of their small size (0.1 to 1.0 μm) and the difficulty in locating them among the overwhelming background of solar system silicates in meteorites. Silicate stardust is more abundant in anhydrous IDPs [450 to 5500 parts per million (ppm)] than in meteorites [<300 parts per billion (ppb) to 180 ppm] (*3*, *10*, *12*–*17*) and micrometeorites (300 ppm) (*9*). Many anhydrous IDPs also harbor discrete μm-sized concentrations of molecular cloud matter, marked by highly elevated D/H and $^{15}N/^{14}N$ ratios (*18*, *19*). The greater survival of presolar materials in anhydrous IDPs shows that these are the least altered remnants of the early solar system, lending support to the view that these IDPs are samples of short-period comets (*20*, *21*).

Here, we report the identification of a silicate grain with a probable origin from a type II supernova. The 500-nm grain (B10A) was identified in five (30 to 70 nm thick) serial sections of an anhydrous cluster IDP (L2011B10) by oxygen isotopic imaging with the Washington University NanoSIMS 50 ion microprobe (*22*). The grain was first identified in O isotopic images of an Au-mounted section, where it was found to have a $^{18}O/^{16}O$ ratio of 13 times the solar value and a $^{17}O/^{16}O$ ratio of one-third that of the solar ratio ($^{18}O/^{16}O = 2.7 \pm 0.1 \times 10^{-2}$, $^{17}O/^{16}O = 1.1 \pm 0.2 \times 10^{-4}$; 1 SD). Simultaneously acquired $^{28}Si^-$ and $^{24}Mg^{16}O^-$ images showed the grain to be a Mg-rich silicate. In an adjacent slice of the IDP, B10A was found to have an anomalous Si isotopic composition [$\delta^{29}Si = -224 \pm 54$ per mil (‰), $\delta^{30}Si = -23 \pm 104$ ‰; 1 SD] (*23*). Because the grain was much smaller in this section (<200 nm), its measured $^{18}O/^{16}O$ ratio ($7.5 \pm 2 \times 10^{-3}$) was significantly influenced by nearby isotopically solar material (*24*). The observed Si isotopic anomaly should thus be considered a lower limit to the grain's true composition. Fe and Mg isotopic measurements were not possible because too little material was available.

Most presolar oxides and silicates fall within four isotopic groups (*25*), pointing to origins in low- to intermediate-mass red giant (RG) and asymptotic giant branch (AGB) stars of differing mass, age, and chemical composition (Fig. 1). In those stars, the onset of abundant dust formation occurs after deep convection brings nucleosynthetically processed material to the stellar surface. The strong ^{17}O enrichments and ^{18}O depletions of group 1 and group 2 grains are due to H burning in the CNO cycle, and the stronger ^{18}O depletions of group 2 grains are ascribed to extra mixing of material at the base of the convective envelope (*26*, *27*). The moderately ^{16}O-rich compositions of group 3 grains are thought to reflect origins from low metallicity RG and AGB stars. The group 4 grains are more enigmatic, but their moderately ^{17}O- and ^{18}O-rich compositions could reflect origins from high metallicity AGB stars. Two exceptional corundum grains have been proposed to originate from type II supernovae (*28*, *29*).

The O isotopic composition of grain B10A falls well outside the range of all previously studied presolar oxides and silicates. The pronounced ^{18}O enrichment is a signature of He burning, where abundant ^{18}O is produced through $^{14}N(\alpha,\gamma)^{18}F(e^+\nu)^{18}O$. The competing reaction $^{18}O(\alpha,\gamma)^{22}Ne$ destroys ^{18}O with increasing efficiency as burning progresses to higher temperatures, resulting in strong variations in the $^{18}O/^{16}O$ ratio of stars of differing mass and age. Abundant ^{12}C is also produced (by 3-α reactions) during He burning, resulting in high C/O ratios at the site where ^{18}O is produced. Although it is possible that ^{18}O-rich material is dredged up during the early evolution of some AGB stars, it must be mixed with the overlying O-rich H envelope to form silicates, leading to a ^{17}O-rich composition instead of the observed ^{17}O depletion. Massive Wolf-Rayet stars may also directly expose ^{18}O-rich products of partial He burning at the stellar surface during the WN-WC transition. However, we do not favor such an origin for this grain because of the high C/O ratio of the ^{18}O-rich ejecta.

The Si isotopic composition of B10A falls near the distribution of X-type SiC grains that have been shown to originate from supernovae (Fig. 2) and is clearly distinct from the far more abundant mainstream SiC grains that derive from RG and AGB stars. Furthermore, the ^{29}Si

[1]Mail Code KR, Robert M. Walker Laboratory for Space Science, NASA Johnson Space Center, Houston, TX 77058, USA. [2]Lunar and Planetary Laboratory, University of Arizona, Tucson, AZ 85721, USA.

*To whom correspondence should be addressed. E-mail: scott.r.messenger@nasa.gov

deficit of B10A is not consistent with an origin from a Wolf-Rayet star, as the ejecta is ^{30}Si and, to a lesser extent, ^{29}Si enriched (*30*).

The O and Si isotopic compositions of B10A point to an origin from a type II supernova. Type II supernovae occur when the core of a massive (>8 $M_\odot$) star collapses and explodes. The structure of a presupernova star consists of concentric compositionally distinct layers undergoing different nucleosynthetic burning stages (*31*), overlain by a massive H-rich convective envelope that experienced partial H burning at its base (*32*). The supernova drives explosive nucleosynthesis in the innermost zones that are subsequently marked by exotic and heterogeneous isotopic compositions. Supernovae produce copious amounts of ^{16}O and thus have low ^{17}O/^{16}O ratios relative to solar ratios (*33*). The relative yield of ^{18}O varies with stellar mass; it is moderately elevated among lower mass supernovae (for M < 15$M_\odot$) and low among higher mass supernovae (*33*).

The isotopic composition of B10A can only be reproduced by a mixture of material from different nucleosynthetic zones. Although B10A has a high ^{18}O/^{16}O ratio (13 times solar), the ^{18}O/^{16}O ratio of the He/C shell is typically 500 to 1000 times solar. Most of the O must have originated from the deeper shells with high relative amounts of ^{16}O (O/C, O/Ne, O/Si, or Si/S), because the low ^{17}O/^{16}O ratio excludes a major contribution from the H/He envelope. Further, the He/C shell is ^{28}Si poor, whereas B10A is ^{28}Si rich. Most of the Si atoms must have originated from the deepest (Si/S, Ni) zones.

TEM investigation found that the material surrounding the supernova grain contains submicrometer forsterite, enstatite, and GEMS grains (glass with embedded metal and sulfides) embedded in carbonaceous material. To unambiguously distinguish which (if any) of the grains were related to the supernova silicate, we imaged this area for $^{16}O^-$, $^{18}O^-$, and $^{32}S^-$, where sulfur was used to mark the location of the GEMS and FeS grains to help align the isotopic and TEM images. As shown in Fig. 3, A to C, the supernova grain is identified as a polycrystalline aggregate of forsterite. The nearby GEMS grains do not share the anomalous isotopic signature, leaving their origins uncertain.

In the thin section studied by TEM in detail, the supernova grain consisted of three discrete elongate lobes <250 nm in size. The lobes are polycrystalline, with well-defined subgrains (50 to 100 nm) that exhibit equilibrium grain boundaries in dark-field images (Fig. 4). Notably, this sub-μm polycrystalline morphology was also recently found in supernova SiC grains (*34*). The subgrains are identified as olivine on the basis of energy-dispersive x-ray analyses showing olivine stoichiometry [Mg/(Mg+Fe) atom ratio of 0.83 ± 0.01] and diffraction spacings characteristic of forsterite. Individual diffraction spots are well defined, with no evidence for streaking or asterism that would indicate extensive disorder (e.g., defects) in the crystalline lattice. We did not observe nuclear particle tracks or substantial (>10 nm) amorphous rims on the grains. There is no apparent crystallographic relationship among the subgrains.

Overall, the microstructure of the grain suggests that the individual crystallites formed separately by condensation before aggregating. The equilibrium grain boundaries between the crystallites are consistent with limited thermal annealing of the grains; however, the thermal event was not long enough or hot enough to completely recrystallize the subgrains. The annealing event may also have erased an earlier radiation history, but the extent of any prior radiation processing was not extensive enough to alter the grain's chemistry substantially.

The identification of B10A as an aggregate of Fe-bearing olivine provides additional constraints on its origin. Many different mixtures of nucleosynthetic zones satisfy the observed isotopic constraints, but most mixtures result in chemical compositions incompatible with olivine condensation. Supernovae are also dynamic, radiation-rich environments that may defy standard views of equilibrium grain condensation. Clayton *et al*. (*35*, *36*) have argued that in supernovae the critical CO molecule is efficiently disrupted by Compton electrons produced by the decay of short-lived nuclides (especially ^{56}Co), resulting in abundant, free neutral C and O atoms. Their model suggests that graphite may condense from such a gas even when C/O < 1 and that oxides may condense from a gas where C/O > 1. Although

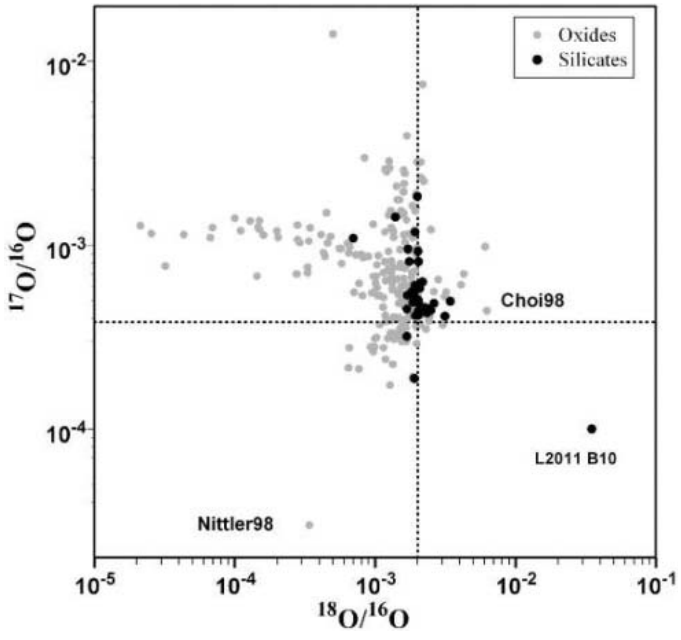


Fig. 1. O isotopic composition of grain B10A compared with previously reported values of presolar corundum (*25*, *28*, *29*, *59*–*61*) and silicates (*3*, *12*–*15*). Two corundum grains identified in the figure as Choi98 and Nittler98 are thought to originate from type II supernovae (*28*, *29*).

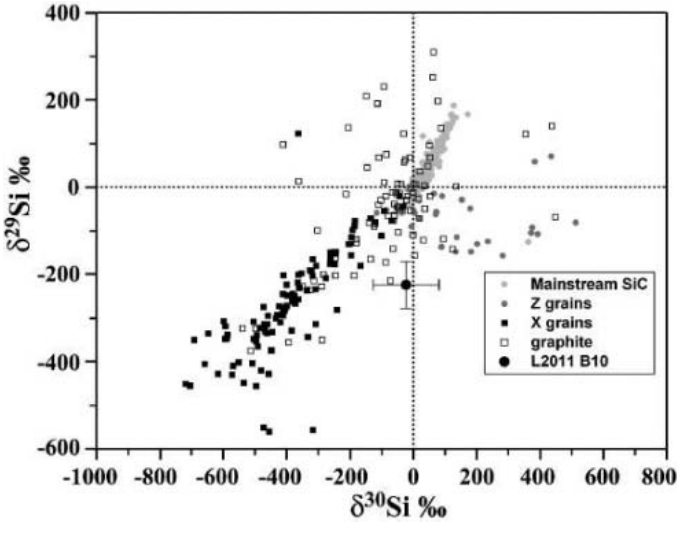


Fig. 2. Si isotopic composition of grain B10A compared with previously analyzed SiC and graphite grains (*62*–*67*). The Si measurement was compromised by surrounding isotopically solar material, and the true composition is likely to be more anomalous. The Si isotopic composition is most similar to X-type SiC grains that are thought to derive from supernovae. The mainstream SiC grains and other minor isotopic subgroups (A, B, Y, and Z grains) account for ~98% of all SiC and are thought to originate from low-mass RG and AGB stars.

B10A is unlikely to have formed from a C-rich gas (based on its $^{18}O/^{16}O$ ratio), the availability of more abundant, free O would have resulted in an enhanced O fugacity that would have helped produce abundant oxidized iron. This unusual circumstance results in an environment more conducive to Fe uptake in condensing olivine (*37*).

Different mixtures of postexplosion zonal compositions were generated from 15 to 25 $M_\odot$ solar metallicity supernovae in appropriate proportions to match the O and Si isotopic composition of B10A (*22*). We explored the equilibrium condensation chemistry of chemical systems with these compositions. In addition, we evaluated the effect of inhibited CO-molecule formation on the condensation chemistry of the system. Our approach is similar to that in (*38*). The system was held at a constant pressure of 10^{-5} bars, and temperature varied from 500 to 2000 K. Thermodynamic data sources and computational techniques are described in (*39*) and (*22*).

From the available nucleosynthetic models, the best match to the isotopic constraints is a mixture comprised of 81 ± 4% He/C zone, 18 ± 1% O/C zone, 0.3 ± 0.02% Si/S zone, and 0.3 ± 0.02% Ni zone of a solar metallicity 15 $M_\odot$ supernova, where the uncertainties reflect ranges in composition consistent with the available constraints. Some elemental abundances of this mixture are distinctly different from those in a solar-composition system. In particular, the H/He ratio is $\sim 10^{-7.2}$, the C/O ratio is 0.89, and C and O are enriched, relative to Si, by factors of 12 and 7, respectively. The abundances of N, Mg, S, Ca, and Fe, relative to Si, are similar to those in a solar-composition system, whereas Al is depleted by ~50%.

Diopside is the first major condensate in this system (*22*). However, olivine is by far the most abundant silicate, condensing at 1560 K as nearly pure forsterite ($Fo_{99.6}$). Lesser amounts of enstatite also form, reaching one-half the olivine abundance. The mole fraction of fayalite in olivine increases at lower temperatures, reaching a maximum value of 0.17 at 1250 K, where the incorporation of Fe into olivine is halted as a result of FeS condensation. The olivine composition remains relatively constant over a large temperature range, decreasing to a value of Fo_{91} at 950 K.

Remarkably, this chemical mixture is an exact match to both the isotopic composition of the grain and the Fe content of the olivine. We were unable to find similarly good matches from any zonal mixtures of higher mass supernovae owing to their lower production of ^{18}O relative to ^{16}O in the He/C shell, but lower mass supernovae may be alternate sources. Our model enables us to make the following inferences for the Mg and Fe isotopic composition of the olivine grain that were unfortunately not possible to measure owing to the small amount of material available: $^{25}Mg/^{24}Mg$ ~12 × solar, $^{26}Mg/^{24}Mg$ ~ 9 × solar, $^{54}Fe/^{56}Fe$ ~ solar, $^{57}Fe/^{56}Fe$ ~ 2 × solar, $^{58}Fe/^{56}Fe$ ~ 8 × solar. The prominently elevated $^{25}Mg/^{24}Mg$ and $^{26}Mg/^{24}Mg$ ratios result from He burning in the O/C shell. The strong enrichment in $^{58}Fe/^{56}Fe$ is a result of slow neutron-capture reactions (s process) in the O/C and He/C shells.

This mixing model and resultant gas composition are not a unique solution, but isotopic constraints require that most of the gas must have been derived from the He/C shell, most (~97%) of the O atoms were derived from interior zones relatively rich in ^{16}O, and a large fraction of the Si atoms originated in the deepest ^{28}Si-rich zones. A substantial contribution of envelope material is also excluded because of its enrichment in $^{17}O/^{16}O$. Furthermore, material from the Si/S and Ni zones must have been mixed into the He/C zone without introducing much of the intervening layers. These conclusions are in line with earlier studies of supernova graphite (*40*), SiC (*41*), and oxide (*29*) grains, whose isotopic compositions could only be reproduced by partial mixing of multiple nucleosynthetic zones.

We also investigated the effect of the radiation-rich environment on the condensation chemistry by inhibiting the formation of CO and of all gas-phase molecules. In this case, SiO was the first phase to condense. The increased O fugacity resulted in olivine condensing at higher temperature and a higher uptake of Fe. However, the gas is so oxidizing that this phase is very unstable, and most of the Fe in the olivine is lost as a result of the condensation of wüstite.

Observations of supernova 1987A showed that there was extensive large-scale mixing of material (*42*), in support of the story told by supernova stardust grains. It is unclear, though, how the large-scale overturn of material results

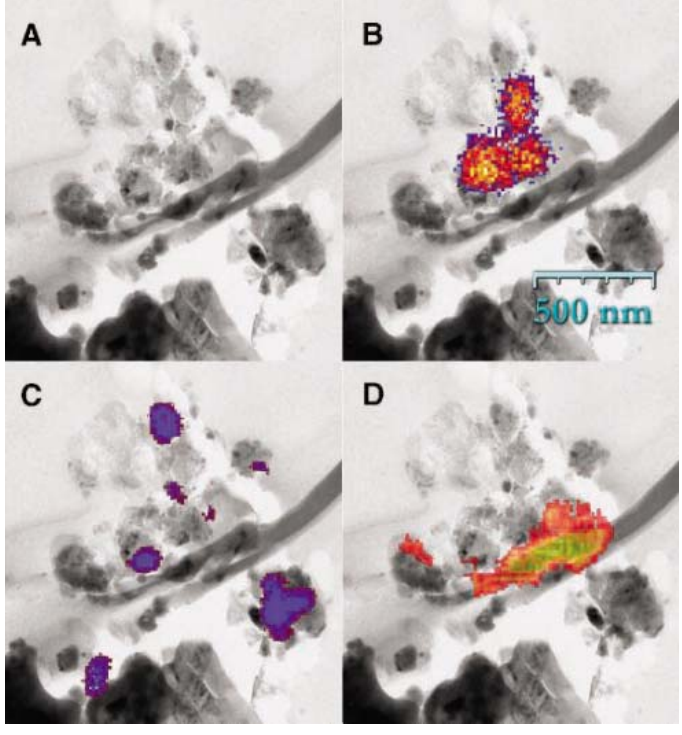


Fig. 3. (**A**) Transmission electron micrograph of a 4 by 4 μm region IDP L2011 B10 found to contain the presolar olivine grain. (**B**) The ^{18}O-rich region of the NanoSIMS isotopic image is overlain, showing that the supernova olivine is made of three distinct lobes. Other portions of the O isotopic image exhibit solar isotopic composition. (**C**) The blue regions show the S hotspots observed by NanoSIMS during the O isotopic image acquisition. These S-rich regions correspond to GEMS grains and small FeS grains. The S-rich spot in the lower left is due to a nearby sulfide; it is not aligned because the thin section stretched upon transfer to the NanoSIMS mount. (**D**) The color overlay shows the location of ^{15}N-rich material as observed in $^{12}C^{14}N$ and $^{12}C^{15}N$ isotopic images.

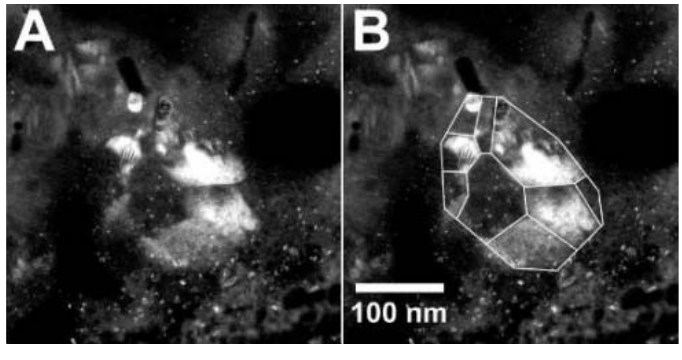


Fig. 4. (**A**) Dark-field TEM image of supernova olivine grain B10A. (**B**) White lines illustrate observed equilibrium grain boundaries between crystallites. Nanometer-sized white specks are due to residual Au coating used to minimize sample charging.

in microscopic mixing of multiple nucleosynthetic zones on a time scale consistent with grain condensation (1 to 2 years).

Deneault *et al*. (*43*) recently suggested that some of this difficulty could be alleviated by implanting material after the grains had formed in reverse shock, possibly accounting for the abundant trace Fe in SiC X grains (*44*). However, this process could not generate the high, uniform abundance of Fe in B10A, because any implanted Fe atoms would have had to remove an equivalent number of Mg atoms to preserve the observed olivine stoichiometry.

Interstellar dust grains accrete coatings of mixed H_2O/organic ices in cold molecular clouds (*45*), and these ices may undergo extensive chemical processing driven by ultraviolet photolysis (e.g., *46*) to become protective coatings of refractory organic matter (*47*). Interestingly, grain B10A is partially embedded in carbonaceous matter. As shown in Fig. 3D, this material has a high $^{15}N/^{14}N$ ratio ($\delta^{15}N = +471 \pm 30$ ‰), like values observed in organic matter in primitive meteorites and IDPs (*48*–*50*). The origin of the ^{15}N enrichment is thought to reflect isotopic fractionation during extremely low-temperature (<20 K) chemical reactions in a presolar cold molecular cloud environment (e.g., *51*). This association of ^{15}N-rich organic matter with stardust has recently been reported for other presolar silicates (*15*), supporting a presolar origin for the N isotopic anomaly.

The well-preserved microstructure of B10A is not easily reconciled with the current understanding of grain processing in the ISM, which is expected to rapidly destroy or render crystalline silicates amorphous. It is possible that the 250-nm-thick layer of presolar organic matter shielded the grain from extensive radiation damage and may also have protected the grain from supernova shocks that act as the primary destruction mechanism for interstellar grains. However, the isotopic signature of the coating is characteristic of a cold molecular cloud environment and thus may not have been present during the grain's initial transit through the diffuse ISM.

Alternatively, this grain may have had an unusually short residence time in the ISM. Interstellar grain lifetimes are estimated to be 200 to 400 million years (*52*, *53*). On the basis of the disparity between the observed abundances of crystalline silicates in evolved stars and the diffuse ISM, crystalline silicates may survive less than 10 million years in this environment (*8*). If this is the case, the parent supernova of B10A may have occurred relatively close to the site of our Sun's formation. The recent demonstration that abundant ^{60}Fe ($t_{1/2}$ = 1.5 million years) was live in the early solar system establishes that there was a late addition of recently synthesized stellar ejecta into the solar nebula—either a type II supernova or an AGB star (*54*, *55*). This must have occurred within a few lifetimes after the ^{60}Fe was produced, consistent with the estimated lifetime of B10A in the diffuse ISM. Although either source is possible, the timing of an AGB star passing near the Sun would have been fortuitous, whereas the solar system is likely to have formed in a massive stellar nursery that was populated by massive O, B stars that quickly evolve and perish as supernovae (*56*).

Because this is the first presolar silicate grain of probable supernova origin among the 132 presolar silicates reported thus far, the relative contribution of supernova silicates appears to be ~1%. This is roughly consistent with the far better known abundance of supernova SiC grains (1%) and Al_2O_3 grains (1%), but much lower than the fraction of presolar graphite thought to originate from supernovae (>30%). These numbers do not support the recent suggestion that type II supernovae are a dominant source of dust in the galaxy, based on observations of the Cassiopeia A supernova remnant (*57*).

References and Notes

1. T. J. Bernatowicz and E. Zinner, Eds., *Astrophysical Implications of the Laboratory Study of Presolar Materials* (AIP Conf. Proc. 402, American Institute of Physics, Woodbury, NY, 1998).
2. E. Zinner, in *Treatise on Geochemistry*, K. K. Turekian, H. D. Holland, v. e. A. M. Davis, Eds. (Elsevier, Oxford, 2004), vol. 1, pp. 17–39.
3. S. Messenger, L. P. Keller, F. J. Stadermann, R. M. Walker, E. Zinner, *Science* **300**, 105 (2003).
4. L. B. F. M. Waters *et al.*, *Astron. Astrophys.* **315**, L361 (1996).
5. F. Kemper, F. J. Molster, C. Jäger, L. B. F. M. Waters, *Astron. Astrophys.* **394**, 679 (2002).
6. F. Kemper, W. J. Vriend, A. G. G. M. Tielens, *Astrophys. J.* **609**, 826 (2004).
7. G. Meeus *et al.*, *Astron. Astrophys.* **365**, 476 (2001).
8. E. F. van Dishoeck, *Annu. Rev. Astron. Astrophys.* **42**, 119 (2004).
9. T. Yada *et al.*, *Lunar Planet. Sci.* **36**, abstr. 1227 (2005).
10. A. Nguyen, E. Zinner, R. Stroud, A. Nguyen, *Lunar Planet. Sci.* **36**, abstr. 2196 (2005).
11. Three of the four amorphous presolar silicate grains found thus far are categorized as GEMS grains (glass with embedded metal and sulfides), materials that comprise a major component of cometary/anhydrous IDPs, occasionally exceeding 50 weight % of the IDP. GEMS grains are typically 0.1 to 0.5 μm in size, have approximately chondritic elemental abundances (with the exception of C), and have optical properties consistent with those observed for interstellar amorphous silicates (*58*).
12. A. Nguyen, E. Zinner, *Science* **303**, 1496 (2004).
13. K. Nagashima, A. N. Krot, H. Yurimoto, *Nature* **428**, 921 (2004).
14. S. Mostefaoui, P. Hoppe, *Astrophys. J.* **613**, L149 (2004).
15. C. Floss, F. J. Stadermann, *Lunar Planet. Sci.* **35**, abstr. 1281 (2004).
16. S. Kobayashi *et al.*, *Lunar Planet Sci.* **36**, abstr. 1931 (2005).
17. P. Hoppe, S. Mostefaoui, T. Stephan, *Lunar Planet. Sci.* **36**, abstr. 1301 (2005).
18. S. Messenger, R. M. Walker, in *Astrophysical Implications of the Laboratory Study of Presolar Materials*, T. J. Bernatowicz, E. Zinner, Eds. (AIP Conf. Proc. 402, American Institute of Physics, Woodbury, NY, 1998), pp. 545–564.
19. S. Messenger, *Nature* **404**, 968 (2000).
20. J. P. Bradley, S. A. Sandford, R. M. Walker, in *Meteorites and the Early Solar System*, J. Kerridge, M. S. Matthews, Eds. (Univ. of Arizona Press, Tucson, AZ, 1988), pp. 861–895.
21. S. Messenger, *Meteorol. Planet. Sci* **37**, 1491 (2002).
22. Materials and methods are available as supporting material on *Science* Online.
23. Delta values represent the deviation of the measured isotopic ratio from the value of the terrestrial standard, in parts per thousand (‰): $\delta R = \left(\frac{R_{measured}}{R_{standard}} - 1\right) \times 1000$.
24. In this case, we acquired $^{16,18}O-$ and $^{28,29,30}Si-$ isotopic images with an estimated spatial resolution of 100 nm. The grain only appeared in the first 3 of the 10 image scans, with an apparent maximum size of 250 nm. By comparing the $^{18}O/^{16}O$ ratio measured in this section (0.0075 ± 0.002, 2 SD) with that of the largest (500 nm) piece ($^{18}O/^{16}O = 0.027 \pm 0.002$, 2 SD), we estimate that nearby material contributed between 69 and 85% of the O ions counted (where the range arises from the uncertainty in the measured $^{18}O/^{16}O$ ratio). If a similar degree of contamination affected the Si data ($\delta^{29}Si = -224 \pm 108$ ‰, $\delta^{30}Si = -23 \pm 208$ ‰; 2 SD), we infer that the true $\delta^{29}Si$ lies between –370 and –1000 ‰. The uncertainty in the $\delta^{30}Si$ value (100 ‰) is too great to permit a similar inference of its true isotopic composition.
25. L. R. Nittler, C. M. O'D. Alexander, X. Gao, R. M. Walker, E. Zinner, *Astrophys. J.* **483**, 475 (1997).
26. A. I. Boothroyd, I.-J. Sackmann, *Astrophys. J.* **510**, 232 (1999).
27. D. D. Clayton, L. R. Nittler, in *Origin and Evolution of the Elements*, A. McWilliam, T. Rauch, Eds. (Carnegie Observatory Astrophysics Series, Pasadena, CA, 2004), pp. 297–316.
28. L. R. Nittler, C. M. O'D. Alexander, J. Wang, X. Gao, *Nature* **393**, 222 (1998).
29. B.-G. Choi, G. R. Huss, G. J. Wasserburg, R. Gallino, *Science* **282**, 1284 (1998).
30. M. Arnould, G. Meynet, G. Paulus, in *Astrophysical Implications of the Laboratory Study of Presolar Materials*, T. J. Bernatowicz, E. Zinner, Eds. (AIP Conf. Proc. 402, American Institute of Physics, Woodbury, NY, 1998), pp. 179–202.
31. B. S. Meyer, T. A. Weaver, S. E. Woosley, *Meteoritics* **30**, 325 (1995).
32. S. E. Woosley, T. A. Weaver, *Astrophys. J. Suppl.* **101**, 181 (1995).
33. T. Rauscher, A. Heger, R. D. Hoffman, S. E. Woosley, *Astrophys. J.* **576**, 323 (2002).
34. R. M. Stroud, L. R. Nittler, P. Hoppe, *Met. Planet. Sci.* **39**, abstr. 5039 (2004).
35. D. D. Clayton, W. Liu, A. Dalgarno, *Science* **283**, 1290 (1999).
36. D. D. Clayton, E. A.-N. Deneault, B. S. Meyer, *Astrophys. J.* **562**, 480 (2001).
37. A. V. Fedkin, L. Grossman, *Lunar Planet. Sci.* **35**, A1823 (2004).
38. D. S. Ebel, L. Grossman, *Geochim. Cosmochim. Acta* **64**, 339 (2000).
39. M. A. Pasek *et al.*, *Icarus* **175**, 1 (2005).
40. C. Travaglio *et al.*, *Astrophys. J.* **510**, 325 (1999).
41. S. Amari, P. Hoppe, E. Zinner, R. S. Lewis, *Astrophys. J.* **394**, L43 (1992).
42. D. H. Wooden, in *Astrophysical Implications of the Laboratory Study of Presolar Materials*, T. J. Bernatowicz, E. Zinner, Eds. (AIP Conf. Proc. 402, American Institute of Physics, Woodbury, NY, 1998), pp. 317–376.
43. E. A.-N. Deneault, D. D. Clayton, A. Heger, *Astrophys. J.* **594**, 312 (2003).
44. K. K. Marhas, P. Hoppe, A. Besmehn, *Lunar Planet. Sci.* **35**, abstr. 1834 (2004).
45. E. L. Gibb *et al.*, *Astrophys. J.* **536**, 347 (2000).
46. M. P. Bernstein, *Astrophys. J.* **576**, 1115 (2002).
47. A. Li, J. M. Greenberg, *Astron. Astrophys.* **323**, 566 (1997).
48. C. M. O'D Alexander *et al.*, *Met. Planet. Sci.* **33**, 603 (1998).
49. L. P. Keller *et al.*, *Geochim. Cosmochim. Acta* **68**, 2577 (2004).
50. C. Floss *et al.*, *Science* **303**, 1355 (2004).
51. S. B. Charnley, S. D. Rodgers, *Astrophys. J.* **569**, L133 (2002).
52. A. P. Jones, A. G. G. M. Tielens, D. J. Hollenbach, *Astrophys. J.* **469**, 740 (1996).
53. H. Mouri, Y. Taniguchi, *Astrophys. J.* **534**, L63 (2000).
54. S. Tachibana, G. R. Huss, *Astrophys. J.* **588**, L41 (2003).
55. M. Busso, R. Gallino, G. J. Wasserburg, *Publ. Astron. Soc. Aust.* **20**, 356 (2003).

56. C. J. Lada, E. A. Lada, *Annu. Rev. Astron. Astrophys.* **41**, 57 (2003).
57. L. Dunne, S. Eales, R. Ivison, H. Morgan, M. Edmunds, *Nature* **424**, 285 (2003).
58. J. P. Bradley *et al.*, *Science* **285**, 1716 (1999).
59. B.-G. Choi, G. J. Wasserburg, G. R. Huss, *Astrophys. J.* **522**, L133 (1999).
60. H. Krestina, W. Hsu, G. J. Wasserburg, *Lunar Planet. Sci.* **33**, abstr. 1425 (2002).
61. E. Zinner *et al.*, *Geochim. Cosmochim. Acta* **67**, 5083 (2004).
62. C. M. O'D. Alexander, *Geochim. Cosmochim. Acta* **57**, 2869 (1993).
63. P. Hoppe, S. Amari, E. Zinner, T. Ireland, R. S. Lewis, *Astrophys. J.* **430**, 870 (1994).
64. P. Hoppe, S. Amari, E. Zinner, R. S. Lewis, *Geochim. Cosmochim. Acta* **60**, 883 (1996).
65. G. R. Huss, I. D. Hutcheon, G. J. Wasserburg, *Geochim. Cosmochim. Acta* **61**, 5117 (1997).
66. S. Amari *et al.*, *Astrophys. J.* **546**, 248 (2001).
67. S. Amari, L. R. Nittler, E. Zinner, K. Lodders, R. S. Lewis, *Astrophys. J.* **559**, 463 (2001).
68. This is the inaugural paper from the newly established Robert M. Walker Laboratory for Space Science at Astromaterials Research and Exploration Science, NASA Johnson Space Center. This research was supported by NASA Cosmochemistry grants to S.M., L.P.K., and D.S.L. This work was enabled by the NanoSIMS ion microprobe designed by G. Slodzian. We thank A. Heger and S. Woosley for generously sharing unpublished supernova model data. The revision of this paper benefited from careful reviews by two anonymous reviewers.

Supporting Online Material
www.sciencemag.org/cgi/content/full/1109602/DC1
Materials and Methods
Figs. S1 and S2
Table S1
References

10 January 2005; accepted 20 June 2005
Published online 30 June 2005;
10.1126/science.1109602
Include this information when citing this paper.

Cytokinin Oxidase Regulates Rice Grain Production

Motoyuki Ashikari,[1]* Hitoshi Sakakibara,[2]* Shaoyang Lin,[3] Toshio Yamamoto,[3] Tomonori Takashi,[3] Asuka Nishimura,[3] Enrique R. Angeles,[3] Qian Qian,[4] Hidemi Kitano,[1] Makoto Matsuoka[1]†

Most agriculturally important traits are regulated by genes known as quantitative trait loci (QTLs) derived from natural allelic variations. We here show that a QTL that increases grain productivity in rice, *Gn1a*, is a gene for cytokinin oxidase/dehydrogenase (OsCKX2), an enzyme that degrades the phytohormone cytokinin. Reduced expression of *OsCKX2* causes cytokinin accumulation in inflorescence meristems and increases the number of reproductive organs, resulting in enhanced grain yield. QTL pyramiding to combine loci for grain number and plant height in the same genetic background generated lines exhibiting both beneficial traits. These results provide a strategy for tailormade crop improvement.

Food shortage is one of the most serious global problems in this century. The United Nations Food and Agricultural Organization (FAO) estimates that 852 million people worldwide were undernourished in 2000 to 2002 (*1*). The global population, now at 6.4 billion, is still growing rapidly and is projected to reach 8.9 billion people by 2050 (*2*). Cereals are an important source of calories for humans, both by direct intake and as the main feed for livestock. About 50% of the calories consumed by the world population originate from three cereals: rice (23%), wheat (17%), and maize (10%) (*3*). To meet the expanding food demands of the rapidly growing world population, crop grain production will need to increase by 50% by 2025 (*4*).

Many agronomically important traits, including yield, are expressed in continuous phenotypic variation. These complex traits usually are governed by a number of genes known as quantitative trait loci (QTLs) derived from natural variations (*5*). QTL analysis has been employed as a powerful approach to discover agronomically useful genes (*6*–*13*).

Rice (*Oryza sativa* L.) is a staple food and has been established as a model monocot because it has the smallest genome size (390 Mb) among the major cereals (*14*), because its genome is syntenic with the genomes of other cereals (*15*), and because rice can be transformed easily. As a result, many molecular markers for rice have been developed, many mutants have been generated and stocked, and the complete genome of rice has been mapped and sequenced (*14*, *16*–*21*). These accomplishments have greatly facilitated QTL analysis in rice. Grain number and plant height are important traits that directly contribute to grain productivity. Dwarf rice and wheat varieties were developed by classical plant breeding methods, contributing to the green revolution in the 1960s. Higher yields were obtained from these dwarf crops because their short stature reduced lodging, which is an agronomic term for bending of plants toward the ground after wind or rain storms (*22*–*25*). During the past decade, many attempts have been made to characterize QTLs for grain production and plant height; however, the genes involved in these QTLs have not been identified yet, and their chromosomal positions remain obscure. We aimed to identify genes of QTLs for grain number and plant height, not only to elucidate molecular mechanisms that regulate grain productivity but also to use these genes for breeding.

QTL analysis. A choice of parental lines that show wide phenotypic variation in the targeted traits is necessary for QTL analysis, because QTL detection is based on natural allelic differences between parental lines. We chose an *indica* rice variety, Habataki, and a *japonica* variety, Koshihikari, because they not only exhibit large variations in agronomically important traits but also have many molecular markers available (*21*). On average, Habataki plants are shorter than individuals of Koshihikari but produce more grains in their main panicle (Fig. 1, A to D).

We developed primary-mapping populations of 96 backcross inbred lines (BILs) derived from the cross between Habataki and Koshihikari. Both grain number and plant height seemed to be regulated by QTLs, as these traits were approximately normally distributed in the mapping population (fig. S1). QTL analysis detected five QTLs for increasing *grain number* (*Gn*) and four QTLs for *plant height* (*Ph*) (Table 1 and Fig. 1E). The most effective QTL for plant height, *Ph1*, was located close to the *semi-dwarf 1* gene (*sd1*) that encodes gibberellin 20 oxidase (*23*–*25*). Comparison of *SD1* between Habataki and Koshihikari revealed that Habataki had a 383–base pair (bp) deletion in the coding region of *gibberellin 20 oxidase*. The resulting loss of function caused the reduced plant height in Habataki. The deletion in the *gibberellin 20 oxidase* is the same as the causal variation found in IR8, a variety that helped lead to the green revolution in rice (*23*–*25*).

The most effective QTL for increasing grain number, *Gn1* on chromosome 1, was selected for further analysis. The Habataki *Gn1* allele is expected to produce ~92 more grains per main panicle than the Koshihikari allele; *Gn1* explains 44% of the difference in grain number between Habataki and Koshihikari (Table 1). So far, several QTLs associated with yield have been reported in rice. Some of these QTLs are located near the *Gn1* region on the short arm of

[1]Bioscience and Biotechnology Center, Nagoya University, Nagoya 464-8601, Japan. [2]Plant Science Center, RIKEN, Tsurumi, Yokohama 230-0045, Japan. [3]Honda Research Institute Japan Co., Ltd., Kisarazu, Chiba, 292-0818 Japan. [4]China National Rice Research Institute, Hangzhou, 310006, China.

*These authors contributed equally to this work.
†To whom correspondence should be addressed. E-mail: makoto@agr.nagoya-u.ac.jp

chromosome 1, suggesting they might be the same QTL (*21*). Although these QTL genes have not been identified and characterized yet, it is possible that the *Gn1* locus contributes to increased grain productivity in various rice varieties. The importance of *Gn1* for enhancing grain number in rice suggested that this QTL would be a good candidate for cloning.

QTL cloning. In QTL cloning, producing nearly isogenic lines (NILs) carrying only one target QTL is necessary to eliminate the effects of other QTLs (*5*). Consequently, the QTL of interest in the NIL can be considered as a single Mendelian factor (*26*). We produced the NIL-*Gn1* carrying the *Gn1* region from Habataki in the Koshihikari background and used it for *Gn1* mapping.

We used 96 F_2 individuals derived from heterozygote (*Gn1/gn1*) plants of NIL-*Gn1* for coarse mapping of *Gn1*. We found that *Gn1* consisted of two loci, QTL-*Gn1a* and QTL-*Gn1b*. QTL-*Gn1a* was mapped within 2 cM between the molecular markers R3192 and C12072S, whereas QTL-*Gn1b* mapped to the upper region of QTL-*Gn1a* (Fig. 1, F and G). *Gn1a* was chosen as the target for positional cloning, because the effects of Habataki *Gn1a* and *Gn1b* loci were almost identical and because the position of *Gn1a* between the two markers had been unambiguously determined. The *Gn1a* allele of Habataki was semidominant, because the grain number of heterozygote plants (*Gn1a/gn1a*) was intermediate between those of homozygote plants, *gn1a/gn1a* and *Gn1a/Gn1a* (Fig. 1J).

About 13,000 F_2 plants derived from heterozygotes (*Gn1a/gn1a*) of NIL-*Gn1a* were used for high resolution mapping of *Gn1a*. The candidate region of *Gn1a* was narrowed down to the 6.3 kb between the markers 3A28 and 3A20 (Fig. 1H). In this region, the Rice Genome Automated Annotation System (*27*) predicted one reading frame with high similarity to cytokinin oxidase/dehydrogenase (CKX), *OsCKX2* (*28*) (Fig. 1H). The *OsCKX2* of Koshihikari and Habataki consist of four exons and three introns and encode proteins of 565 or 563 amino acids, respectively. Comparison of the DNA sequences between the cultivars revealed several nucleotide changes, including a 16-bp deletion in the 5′-untranslated region, a 6-bp deletion in the first exon, and three nucleotide changes resulting in amino acid variation in the first and fourth exons of the Habataki allele (Fig. 1I).

We also analyzed the nucleotide sequences of *OsCKX2* in three alleles of high-yielding rice varieties from China, 5030, 5150, and 90B2. An 11-bp deletion in the coding region was detected in 5150, which produced more than 400 grains in the main panicle in our experimental field (Fig. 1, C and D). This deletion created a premature stop codon, suggesting that 5150 is null for *OsCKX2* (Fig. 1I). The other two varieties had sequences identical to the Habataki allele. The coincidence of the *OsCKX2* null allele and a higher grain number suggested that a reduction or loss of function of OsCKX2 enhanced grain production.

To confirm that *OsCKX2* corresponds to *Gn1a*, we produced transgenic plants expressing different levels of *OsCKX2* and examined their grain yield. As Koshihikari and Habataki fail to regenerate shoots from the callus, we used the easily regenerable cultivar Taichung 65 (TC65), which possesses the Koshihikari allele of *OsCKX2*. Transgenic plants carrying two copies of the sense strand of *OsCKX2* that

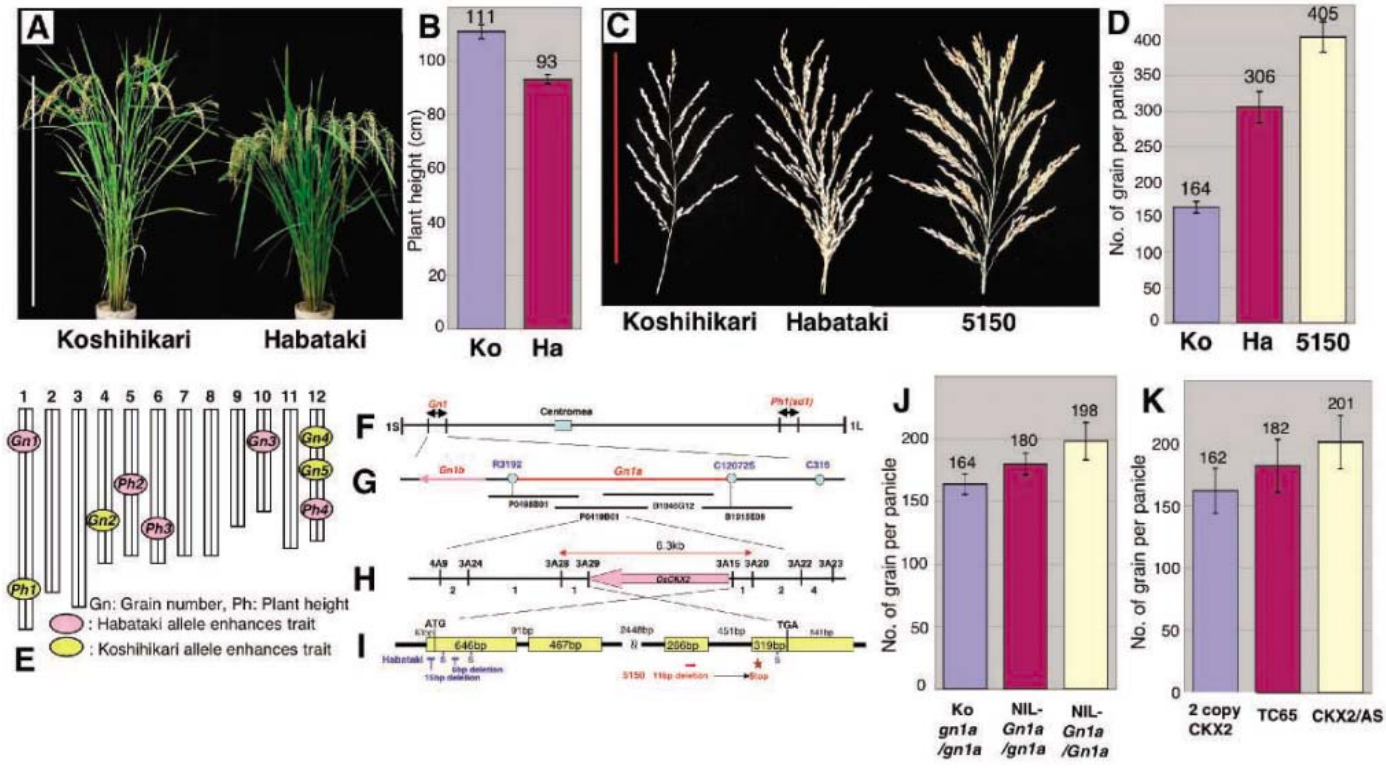


Fig. 1. QTL analysis and molecular cloning. (**A**) Gross morphology of Koshihikari and Habataki at maturity. Scale bar, 1 m. (**B**) Comparison of plant height at the heading stage in Koshihikari (Ko) and Habataki (Ha). (**C**) Panicle structure of Koshihikari, Habataki, and 5150. Scale bar, 20 cm. (**D**) Comparison of grain number in the main panicle of Koshihikari (Ko), Habataki (Ha), and 5150. Values in (B) and (D) are means with SD (n = 10 plants). (**E**) QTL map of grain number (*Gn*) and plant height (*Ph*) on rice chromosomes. (**F**) Location of *Gn1* and *Ph1* on chromosome 1. (**G**) Coarse linkage map and physical map of *Gn1*. (**H**) High-resolution linkage map of *Gn1a*. The number of recombinants between the molecular markers is indicated below the high-resolution map. (**I**) *OsCKX2* structure and mutation sites in Habataki (blue) and 5150 (red). S indicates the site of amino acid substitutions. (**J**) Comparison of grain number per main panicle in Koshihikari (Ko-*gn1a/gn1a*), NIL-*Gn1a/gn1a*, and NIL-*Gn1a/Gn1a*. (**K**) Comparison of grain number per main panicle in nontransgenic and transgenic lines. 2 copy CKX2, transgenic TC65 plant carrying two copies of *OsCKX2* derived from Koshihikari; TC65, control *japonica* line; CKX2/AS, transgenic rice carrying antisense *OsCKX2* cDNA from Koshihikari. Values in (J) and (K) are means with SD (n = 10 plants).

was highly expressed showed reduced grain numbers compared to TC65. However, transgenic plants with antisense strands of *OsCKX2* that had reduced levels of expression developed higher grain numbers (Fig. 1K and fig. S2). We conclude that the QTL for increased grain number, *Gn1a*, is *OsCKX2*.

Molecular analysis of OsCKX2. Cytokinin (CK) was first discovered as a plant hormone that promotes cell division (*29*). It is now known to influence various aspects of plant growth and development, including seed germination, apical dominance, leaf expansion, reproductive development, and delay of senescence (*30*). Natural CKs such as *trans*-zeatin (tZ) and isopentenyladenine (iP) are N^6-substituted adenine derivatives that generally contain an isoprenoid side chain (*31*). CKX preferentially and irreversibly degrades nucleobase CKs by cleavage of the unsaturated N^6-isoprenoid side chains (*31*). This catabolic enzyme probably plays the principal role in controlling CK levels in plant tissues (*32*–*34*).

To examine whether the *OsCKX2* locus affects CK metabolism, we analyzed the levels of CKs in inflorescence meristems of Koshihikari, Habataki, NIL-*Gn1a*, and 5150. Although the contents of active tZ were similar in these lines, CK nucleotides (i.e., tZRMP and iPRMP) were substantially more abundant in Habataki, NIL-*Gn1a*, and 5150 than in Koshihikari (Fig. 2A). Because the CK metabolism modifying the adenine moiety is partially shared with the purine salvage pathway, nucleobase CKs are readily converted to the corresponding nucleotides and nucleosides (*31*). This metabolic flow plays an important role in the homeostasis of active CKs (*31*). In this context, the accumulation of the nucleotide- and nucleoside-species is explainable by the reduction in CKX activity in Habataki, NIL-*Gn1a*, and 5150, and the increased production of CK conjugates to reduce the overall CK activity (see below).

To test whether *OsCKX2* encodes an active enzyme in Koshihikari and Habataki, we isolated the cDNAs and expressed the proteins in budding yeast, *Saccharomyces cerevisiae*. Although several amino acids varied between the two OsCKX2 proteins, they catalyzed the cleavage of iP side chains with similar specific ac-

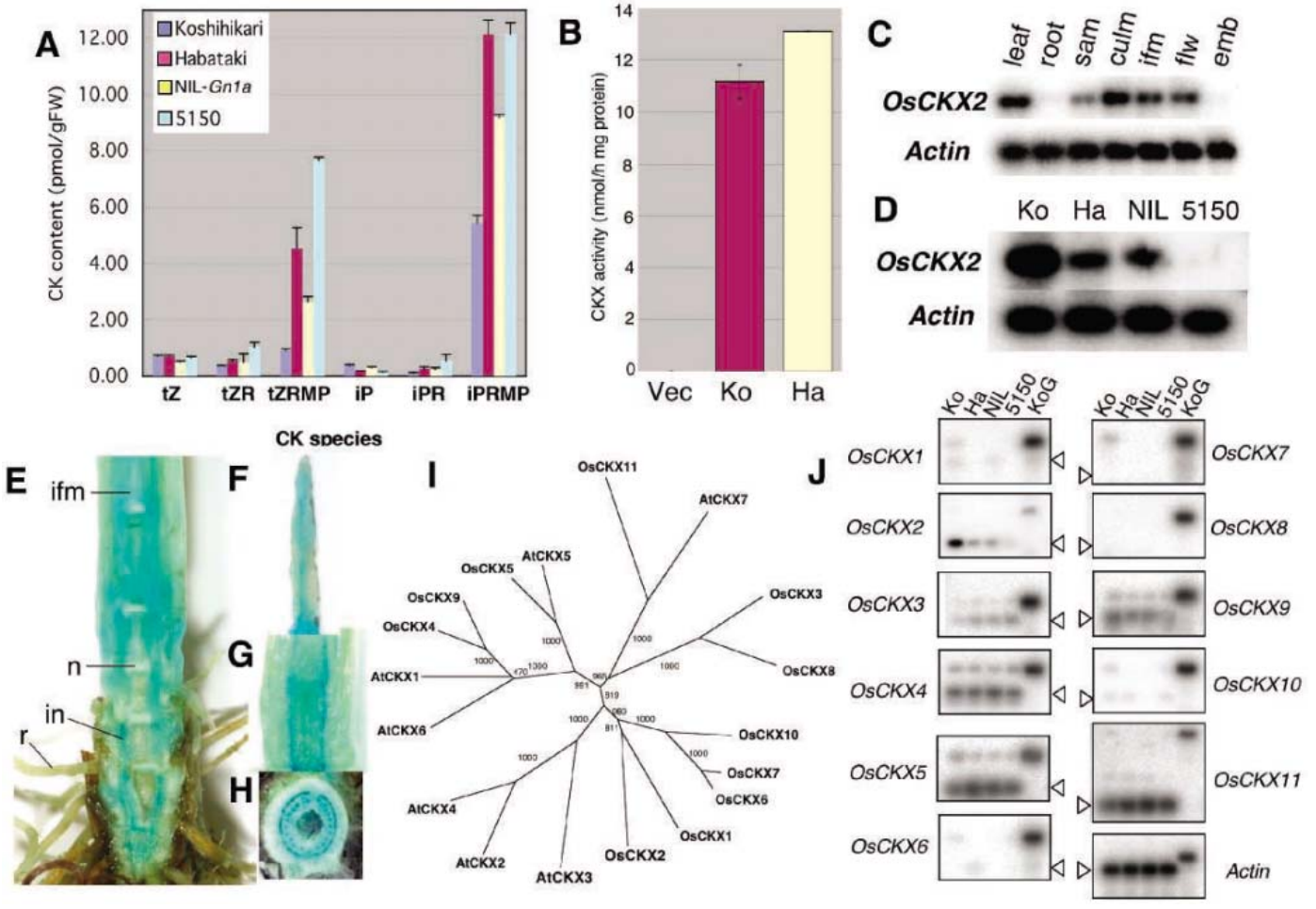


Fig. 2. Molecular characterization of *OsCKX2*. (**A**) Comparison of CK levels in the inflorescence meristem of Koshihikari, Habataki, NIL-*Gn1a*, and 5150. tZ, *trans*-zeatin; tZR, tZ riboside; tZRMP, tZR 5′-monophosphate; iP, isopentenyladenine; iPR, iP riboside; iPRMP, iPR 5′-monophosphate; gFW, grams fresh weight. Values are means with SD (n = 3 measurements). (**B**) Enzymatic CKX activity in yeast cells transformed with empty vector (Vec), the *OsCKX2* allele from Koshihikari (Ko), and that from Habataki (Ha). (**C**) Expression analysis by RT–Southern blot of *OsCKX2* in various organs [leaf, root, shoot apex meristem (sam), culm, inflorescent meristem (ifm), flower (flw), and embryo (emb)] of rice. (**D**) *OsCKX2* expression in the inflorescence meristem of Koshihikari (Ko), Habataki (Ha), NIL-*Gn1a* (NIL), and 5150. Actin was used as a control in (C) and (D). (**E** to **H**) GUS expression under the control of the *OsCKX2* promoter: (E) longitudinal section of a culm (ifm, inflorescent meristem; n, node; in, internode; r, root); (F) young flower; (G) longitudinal section of node and internode; and (H) transverse section of internode. (**I**) Phylogenetic relationship of CKX proteins in rice and *Arabidopsis*. OsCKXs, *O. sativa* CKXs (table S1); AtCKXs, *Arabidopsis* CKXs (*28*). (**J**) Expression analysis of *OsCKX1* to *OsCKX11* in the inflorescence meristem of Koshihikari (Ko), Habataki (Ha), NIL-*Gn1a* (NIL), and 5150 by RT–Southern blot. The Koshihikari genomic DNA was used as a template for the positive control (KoG) in the polymerase chain reaction (PCR) with primers designed for each *OsCKX*. The PCR produced bands at higher molecular weights than those generated from cDNA because they contained intron sequences. The expected signal sizes of the cDNAs are indicated by arrowheads.

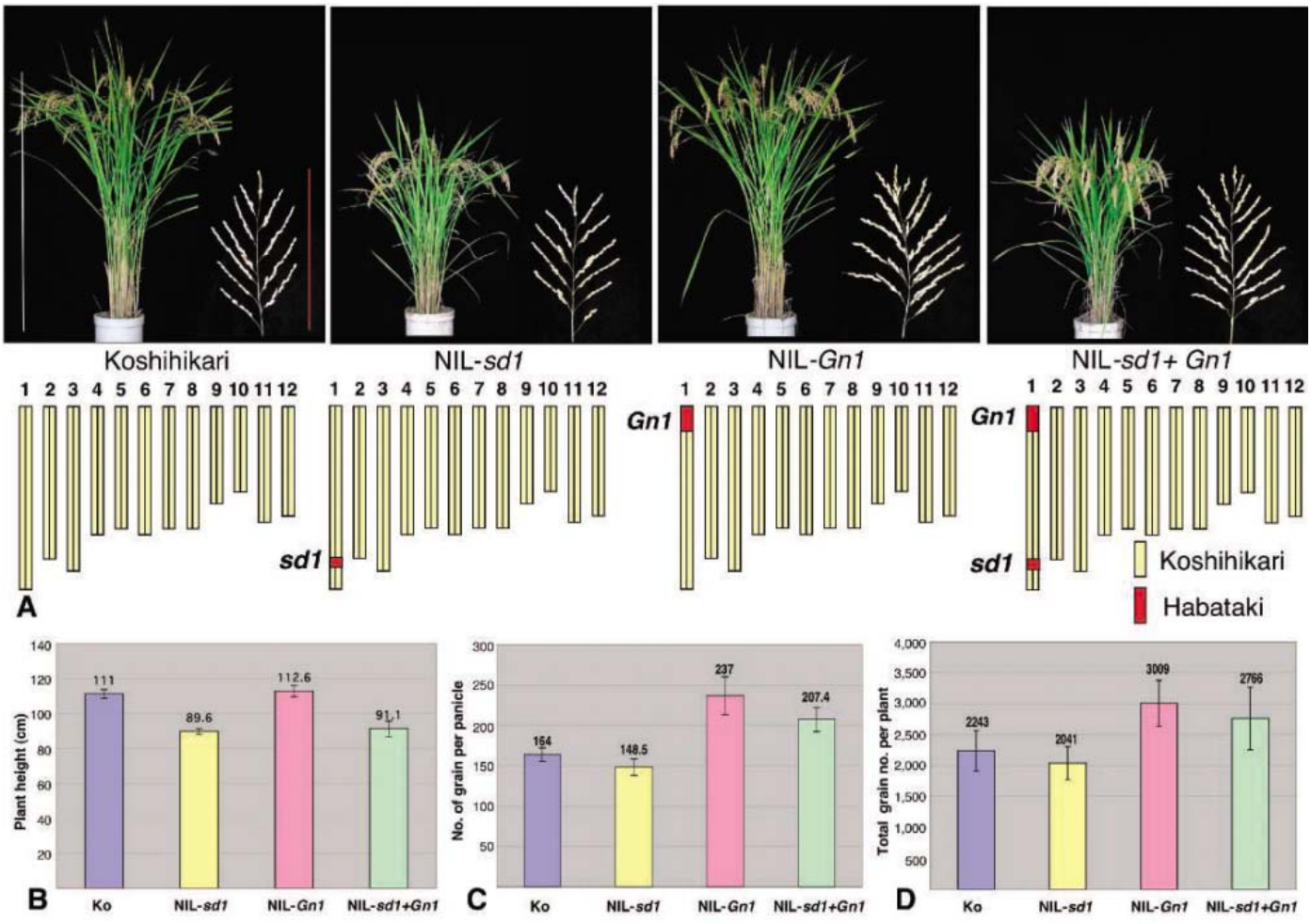


Fig. 3. Phenotypic characterization of NIL-*QTLs*. (**A**) Plant morphologies and chromosome maps of Koshihikari, NIL-*sd1*, NIL-*Gn1*, and NIL-*sd1*+*Gn1*. White and red scale bars indicate 1 m and 20 cm, respectively. (**B**) Comparison of plant height, (**C**) grain number in the main panicle, and (**D**) grain number in whole plants for Koshihikari, NIL-*sd1*, NIL-*Gn1*, and NIL-*sd1*+*Gn1*. Values in (B) to (D) are means with SD (n = 10 plants).

tivities (Fig. 2B). This result shows that both alleles of *OsCKX2* encode functional enzymes.

We next studied the expression profiles of *OsCKX2* in various organs by reverse transcription (RT)–Southern blotting, because RNA gel blot analysis did not detect any signals because of the low expression levels. The RT–Southern blot showed that *OsCKX2* was preferentially expressed in leaves, culms, inflorescence meristems, and flowers (Fig. 2C). The highest levels of *OsCKX2* expression in inflorescence meristems were found in Koshihikari. Transcript accumulation was less abundant in Habataki and NIL-*Gn1a* and extremely low in 5150 (Fig. 2D). As these differences indicated a correlation between *OsCKX2* expression levels and grain number, they suggested that the phenotypic differences observed might have been caused by differential transcription of *OsCKX2*.

We next examined the tissue specificity of *OsCKX2* expression in transgenic rice harboring an *OsCKX2 promoter*::*β-glucuronidase* (GUS) construct. GUS expression was observed mainly in the vascular tissue in developing culms, inflorescence meristems, and young flowers in the T2 generation of transgenic plants (Fig. 2, E to H). The expression of *OsCKX2* in inflorescence meristems might regulate the CK level to control flower number. CK is known to be translocated acropetally via the xylem and systemically via the phloem (*35*). The high levels of expression in these tissues suggest that *OsCKX2* plays a role in regulating CK levels in the vascular system of developing culms, where CK is transported to the inflorescence meristems.

At least 11 putative *CKX* genes (*OsCKX1* to *OsCKX11*) are present in the rice genome (Fig. 2I and table S1). This redundancy suggests that the *OsCKXs* could be functionally differentiated by their temporal and spatial expression patterns. In tobacco and *Arabidopsis*, overexpression of *CKX* results in reduced levels of endogenous CK and lower meristem activity (*33*, *34*). In transgenic *Arabidopsis*, overexpression of *AtCKX3*, the allele showing the highest similarity to *OsCKX2* of the seven *AtCKXs* (Fig. 2I), reduced flower number because of a decreased rate of primordia formation in the flower meristem (*34*). Our findings are in agreement with these results from *Arabidopsis*.

We examined the expression of all *OsCKX* genes in the inflorescence meristems of the four lines, Koshihikari, Habataki, NIL-*Gn1a*, and 5150, to elucidate why their phenotypes were different despite the apparent high redundancy of *OsCKX* genes in the rice genome. *OsCKX2* was the dominant *OsCKX* expressed in Koshihikari inflorescence meristems (Fig. 2J), underlining its role in crop productivity. In contrast to *OsCKX2*, the expression patterns of all other *OsCKX* genes did not differ among these cultivars (Fig. 2J), indicating that OsCKX2 functions in inflorescence development.

QTL pyramiding. In this experiment, *Gn1a* was identified as *OsCKX2*, a gene that increased grain number by ~21% (Fig. 1J). *Gn1b*, another QTL, has not been identified yet and will be the next target for characterization. The Habataki alleles of *Gn1a* and *Gn1b* additively increase grain number; both components of *Gn1* (*Gn1a* and *Gn1b*) are ideally suited for application to a practical breeding program. The NIL-*Gn1* carrying the *Gn1* locus

Table 1. Putative QTLs for grain number (*Gn*) and plant height (*Ph*). QTL names are designated with the abbreviation of the trait name. NML, nearest marker locus of putative QTLs; PVE, phenotypic variation explained by each QTL; LOD, logarithm of odds.

QTL name	Chromosome number	NML	Position of NML (cM)	Change in effect*	PVE	LOD
			Grain number			
Gn1	1	BB-85	22.6	H92	44%	9.863
Gn2	4	AE-19	102.1	K41	10%	1.922
Gn3	10	AJ-65	30.2	H39	7%	1.38
Gn4	12	BI-20	30	K35	9%	1.701
Gn5	12	BB-23	47	K35	11%	2.147
			Plant height			
Ph1	1	BC-55	146.4	K24	30%	6.531
Ph2	5	BF-37	66.5	H10	7%	1.389
Ph3	6	CC-84	107.3	H11	9%	1.629
Ph4	12	BB-48	91.4	H9	8%	1.565

*H indicates a Habataki-enhanced trait; K, a Koshihikari-enhanced trait. In grain numbers or cm of plant height.

(*Gn1a* and *Gn1b*) of Habataki has a heavier panicle weight and is more susceptible to lodging.

To resolve the problem, we employed a QTL pyramiding breeding strategy. In this approach, desirable QTLs are combined through crossing of NIL-*QTLs* into a common genetic background. First, we developed an NIL carrying the Habataki *sd1* allele in the Koshihikari background (Fig. 3A). This NIL-*sd1* was ~20% shorter than Koshihikari, as expected because of the effect of the *sd1* allele (Fig. 3, A and B). Simultaneously, an NIL-*Gn1* carrying the Habataki *Gn1a*+*Gn1b* chromosome fragment that produced ~45% more grain than Koshihikari (Fig. 3, A and C) was also selected. The degrees of increase in grain number (45%) and reduction of plant height (20%) in the NILs corresponded to the phenotypic variation effects of *Gn1* (44%) or *Ph1* (30%), respectively, as predicted by the QTL analysis (Table 1). NIL-*sd1*+*Gn1* was generated by crossing NIL-*Gn1* and NIL-*sd1* (Fig. 3A). The grain number in the main panicle was 26% higher, and plants were 18% shorter in this line than in Koshihikari (Fig. 3, A to C). The reduction in grain number for NIL-*sd1*+*Gn1* (207 grains), as compared to NIL-*Gn1* (237 grains), seemed due to pleiotropic effects of the *sd1* allele. The same degree of grain number reduction was also found in NIL-*sd1*, relative to Koshihikari (Fig. 3C). The positive effect of *Gn1*, however, outweighed the negative effect of *sd1*, in NIL-*sd1*+*Gn1* (Fig. 3C). Because total grain number per plant is the most important factor for increasing grain yield under field production conditions, grain numbers per plant were compared rather than grain numbers per main panicle. When the comparison was based on grain numbers per plant, 34% and 23% increases were found in NIL-*Gn1* and NIL-*sd1*+*Gn1*, respectively (Fig. 3D). No difference in grain size was observed for these two NILs. Thus, *Gn1* is a useful locus for increasing grain productivity.

Toward the application of QTLs for a new green revolution. We succeeded in cloning a QTL (*Gn1a*) that increased grain number in rice. *Gn1a* encodes OsCKX2, an enzyme that degrades bioactive CK. A null allele of *OsCKX2* had been selected for increasing crop yield in a conventional breeding program in China. Genome synteny allows breeders to integrate traits and genes among cereals (*15*). For example, rice chromosome 1 shows regions of sequence similarity with chromosomes 3, 6, and 8 in maize (*36*), where some QTLs for grain yield traits have been mapped (*37*–*40*). *Gn1a* in rice might correspond to these maize QTLs, and orthologous CKX genes in other species might regulate yield in other cereal crops as in rice.

In molecular studies of rice and wheat varieties, the phytohormone gibberellin has been identified as a key player in controlling crop plant architecture (*22*–*25*). We demonstrate here that CK metabolism also contributes to crop productivity. Because CK controls cell division and lateral meristem activity (*30*, *31*), CK accumulation in the inflorescence meristem can explain the significantly higher grain numbers.

Identification of agronomically important QTLs and pyramiding of such QTLs presents a useful strategy for efficient crop breeding. Interspecific crosses between *O. sativa* and wild relatives could lead to the discovery of useful QTLs from a range of allelic variations much wider than those present in cultivated lines. Furthermore, wild rice species are likely to provide access to QTLs not only for yield but also for disease resistance, stress tolerance, and other desirable traits (*6*–*8*, *21*), because these plants have adapted to unique geographic and environmental conditions. Discovering useful genes, improving agricultural traits hidden in the plant genome, and applying these findings to crop breeding will pave the way for a new green revolution.

References and Notes

1. FAO, "The state of food insecurity in the world," available at www.fao.org/documents/show_cdr.asp?url_file=/docrep/007/y5650e/y5650e00.htm (2004).
2. United Nations Population Fund, "State of the world population," available at www.unfpa.org/swp/2004/english/ch1/page7.htm (2004).
3. G. S. Khush, *Nutr. Rev.* **61**, 114 (2003).
4. G. S. Khush, *Proc. Nutr. Soc.* **60**, 15 (2001).
5. M. Yano, *Curr. Opin. Plant Biol.* **4**, 130 (2001).
6. J. Doebley, A. Stec, L. Hubbard, *Nature* **386**, 485 (1997).
7. E. Fridman, T. Pleban, D. Zamir, *Proc. Natl. Acad. Sci. U.S.A.* **97**, 4718 (2000).
8. A. Frary *et al.*, *Science* **289**, 85 (2000).
9. M. Yano *et al.*, *Plant Cell* **12**, 2473 (2000).
10. S. E. D. El-Assal *et al.*, *Nat. Genet.* **29**, 435 (2001).
11. J. Liu, J. Van Eck, B. Cong, S. D. Tanksley, *Proc. Natl. Acad. Sci. U.S.A.* **99**, 13302 (2002).
12. Y. Takahashi, A. Shomura, T. Sasaki, M. Yano, *Proc. Natl. Acad. Sci. U.S.A.* **98**, 7922 (2001).
13. S. Kojima *et al.*, *Plant Cell Physiol.* **43**, 1096 (2002).
14. T. Sasaki, T. Matsumoto, B. A. Antonio, Y. Nagamura, *Plant Cell Physiol.* **46**, 3 (2005).
15. M. D. Gale, K. M. Devos, *Proc. Natl. Acad. Sci. U.S.A.* **95**, 1971 (1998).
16. J. Yu *et al.*, *Science* **296**, 79 (2002).
17. S. A. Goff *et al.*, *Science* **296**, 92 (2002).
18. T. Sasaki *et al.*, *Nature* **420**, 312 (2002).
19. Q. Feng *et al.*, *Nature* **420**, 316 (2002).
20. The Rice Chromosome 10 Sequencing Consortium, *Science* **300**, 1566 (2003).
21. Materials and methods are available as supporting material on *Science* Online.
22. J. R. Peng *et al.*, *Nature* **400**, 256 (1999).
23. A. Sasaki *et al.*, *Nature* **416**, 701 (2002).
24. M. Ashikari *et al.*, *Breed. Sci.* (2002).
25. W. Spielmeyer, M. H. Ellis, P. M. Chandler, *Proc. Natl. Acad. Sci. U.S.A.* **99**, 9043 (2002).
26. A. H. Paterson *et al.*, *Nature* **335**, 721 (1988).
27. Rice Genome Automated Annotation System, available at http://RiceGAAS.dna.affrc.go.jp/.
28. T. Schmülling *et al.*, *J. Plant Res.* **116**, 241 (2003).
29. C. O. Miller, F. Skoog, M. H. Vonsaltza, F. M. Strong, *J. Am. Chem. Soc.* **77**, 1392 (1955).
30. M. C. Mok, in *Cytokinin: Chemistry, Activity, and Function*, D. W. S. Mok, M. C. Mok, Eds. (CRC Press, Boca Raton, FL, 1994), ch. 12.
31. D. W. S. Mok, M. C. Mok, *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **52**, 89 (2001).
32. D. Armstrong, in *Cytokinin: Chemistry, Activity, and Function*, D. W. S. Mok, M. C. Mok, Eds. (CRC Press, Boca Raton, FL, 1994), ch. 11.
33. T. Werner, V. Motyka, M. Strnad, T. Schmülling, *Proc. Natl. Acad. Sci. U.S.A.* **98**, 10487 (2001).
34. T. Werner *et al.*, *Plant Cell* **15**, 2532 (2003).
35. G. Bernier *et al.*, *Plant Cell* **5**, 1147 (1993).
36. J. Salse, B. Piegu, R. Cooke, M. Delseny, *Plant J.* **38**, 396 (2004).
37. L. R. Veldboom, M. Lee, *Crop Sci.* **36**, 1310 (1996).
38. D. F. Austin, M. Lee, L. R. Veldboom, A. R. Hallauer, *Crop Sci.* **40**, 30 (2000).
39. J. C. Ho, S. R. McCouch, M. E. Smith, *Theor. Appl. Genet.* **105**, 440 (2002).
40. Maize Genetics and Genomics Database, available at www.maizegdb.org.

41. We thank N. Itoh (Nagoya University) for managing plant materials and technical assistance; N. Watanabe, Y. Kondoh, and M. Tatsuki (Honda Research Institute Japan) for helpful suggestions in the experimental design; and M. Kojima and Y. Mukouzaka (RIKEN) for their technical assistance. Supported by Honda Research Institute Japan.

Supporting Online Material

www.sciencemag.org/cgi/content/full/1113373/DC1
Materials and Methods
Figs. S1 and S2
Table S1
References and Notes

11 April 2005; accepted 23 May 2005
Published online 23 June 2005;
10.1126/science.1113373
Include this information when citing this paper.

Discovery of Very High Energy Gamma Rays Associated with an X-ray Binary

F. Aharonian,[1] A. G. Akhperjanian,[2] K.-M. Aye,[3] A. R. Bazer-Bachi,[4] M. Beilicke,[5] W. Benbow,[1] D. Berge,[1] P. Berghaus,[6]* K. Bernlöhr,[1,7] C. Boisson,[8] O. Bolz,[1] V. Borrel,[4] I. Braun,[1] F. Breitling,[7] A. M. Brown,[3] J. Bussons Gordo,[9] P. M. Chadwick,[3] L.-M. Chounet,[10] R. Cornils,[5] L. Costamante,[1] B. Degrange,[10] H. J. Dickinson,[3] A. Djannati-Ataï,[6] L. O'C. Drury,[11] G. Dubus,[10]† D. Emmanoulopoulos,[12] P. Espigat,[6] F. Feinstein,[9] P. Fleury,[10] G. Fontaine,[10] Y. Fuchs,[13] S. Funk,[1] Y. A. Gallant,[9] B. Giebels,[10] S. Gillessen,[1] J. F. Glicenstein,[14] P. Goret,[14] C. Hadjichristidis,[3] M. Hauser,[12] G. Heinzelmann,[5] G. Henri,[13] G. Hermann,[1] J. A. Hinton,[1] W. Hofmann,[1] M. Holleran,[15] D. Horns,[1] A. Jacholkowska,[9] O. C. de Jager,[15] B. Khélifi,[1] Nu. Komin,[7] A. Konopelko,[1,7] I. J. Latham,[3] R. Le Gallou,[3] A. Lemière,[6] M. Lemoine-Goumard,[10] N. Leroy,[10] T. Lohse,[7] A. Marcowith,[4] J.-M. Martin,[8] O. Martineau-Huynh,[16] C. Masterson,[1] T. J. L. McComb,[3] M. de Naurois,[16]† S. J. Nolan,[3] A. Noutsos,[3] K. J. Orford,[3] J. L. Osborne,[3] M. Ouchrif,[16] M. Panter,[1] G. Pelletier,[13] S. Pita,[6] G. Pühlhofer,[1,12] M. Punch,[6] B. C. Raubenheimer,[15] M. Raue,[5] J. Raux,[16] S. M. Rayner,[3] A. Reimer,[17] O. Reimer,[17] J. Ripken,[5] L. Rob,[18] L. Rolland,[16] G. Rowell,[1] V. Sahakian,[2] L. Saugé,[13] S. Schlenker,[7] R. Schlickeiser,[17] C. Schuster,[17] U. Schwanke,[7] M. Siewert,[17] H. Sol,[8] D. Spangler,[3] R. Steenkamp,[19] C. Stegmann,[7] J.-P. Tavernet,[16] R. Terrier,[6] C. G. Théoret,[6] M. Tluczykont,[10] G. Vasileiadis,[9] C. Venter,[15] P. Vincent,[16] H. J. Völk,[1] S. J. Wagner[12]

X-ray binaries are composed of a normal star in orbit around a neutron star or stellar-mass black hole. Radio and x-ray observations have led to the presumption that some x-ray binaries called microquasars behave as scaled-down active galactic nuclei. Microquasars have resolved radio emission that is thought to arise from a relativistic outflow akin to active galactic nuclei jets, in which particles can be accelerated to large energies. Very high energy γ-rays produced by the interactions of these particles have been observed from several active galactic nuclei. Using the High Energy Stereoscopic System, we find evidence for gamma-ray emission of >100 gigaelectron volts from a candidate microquasar, LS 5039, showing that particles are also accelerated to very high energies in these systems.

High-resolution radio maps of x-ray binaries (XRBs) have revealed powerful outflows that are similar to the relativistic jets seen in active galactic nuclei (AGN) (*1*, *2*). In both cases, the radio emission is due to synchrotron radiation from particles accelerated to high energies. These outflows probably result from the accretion of material onto the compact object, albeit on vastly different scales: The mass (size) of black holes in AGN is at least 10^6 times that of compact objects in XRBs. Hence, XRBs with resolved radio emission have been dubbed microquasars, reflecting the suspicion of some fundamental scaling with compact object mass.

The kinship should be most evident close to the black hole, where the jet is launched and where the available energy reservoir to accelerate particles is largest. In AGN the particles can reach energies such that their nonthermal emission extends to the GeV-TeV γ-ray regime, via Compton upscattering of ambient photons or as a result of high-energy hadron interactions. Because of relativistic bulk motion, this emission is most easily seen in blazars, where the AGN jet is aligned close to the line of sight. Very high energy (VHE) γ-rays are to be expected from some XRBs if the physical processes in the vicinity of the compact object are indeed analogous. However, previous observations of VHE emission from XRBs were inconclusive (*3*).

Two XRBs have resolved radio emission in the 0.001 arc sec range, which is presumed to be associated with a relativistic jet, and possible counterparts in the MeV-GeV domain (*4*–*6*). LS 5039 (RX J1826.2-1450) and LSI +61°303 (V615 Cas) are each composed of a massive star in an eccentric orbit around an undetermined compact object (*7*, *8*). Their proposed γ-ray counterparts are respectively localized to 0.5° (3EG J1824-1514) and 0.2° (3EG J0241+6103) in the Third Energetic Gamma Ray Experiment Telescope (EGRET) catalog (*9*), so that the association cannot be considered firm on the basis of positional coincidence alone. The systems are quite inconspicuous in x-rays, with low variability and luminosity of $\sim 10^{34}$ erg s^{-1} at 1 to 10 keV (*10*, *11*), which is about one-tenth their luminosity above 100 MeV, assuming that the EGRET sources are indeed counterparts. The γ-ray spectra measured by EGRET are hard,

[1]Max-Planck-Institut für Kernphysik, Post Office Box 103980, D-69029 Heidelberg, Germany. [2]Yerevan Physics Institute, 2 Alikhanian Brothers Street, 375036 Yerevan, Armenia. [3]Department of Physics, University of Durham, South Road, Durham DH1 3LE, UK. [4]Centre d'Etude Spatiale des Rayonnements, CNRS/UPS, 9 av. du Colonel Roche, BP 4346, F-31029 Toulouse Cedex 4, France. [5]Institut für Experimentalphysik, Universität Hamburg, Luruper Chaussee 149, D-22761 Hamburg, Germany. [6]APC, UMR 7164 du CNRS, 11 Place Marcelin Berthelot, F-75231 Paris Cedex 05, France. [7]Institut für Physik, Humboldt-Universität zu Berlin, Newtonstr. 15, D-12489 Berlin, Germany. [8]Laboratoire Univers et Théories (LUTH), UMR 8102 du CNRS, Observatoire de Paris, Section de Meudon, F-92195 Meudon Cedex, France. [9]Laboratoire de Physique Théorique et Astroparticules, IN2P3/CNRS, Université Montpellier II, CC 70, Place Eugène Bataillon, F-34095 Montpellier Cedex 5, France. [10]Laboratoire Leprince-Ringuet, IN2P3/CNRS, Ecole Polytechnique, F-91128 Palaiseau, France. [11]Dublin Institute for Advanced Studies, 5 Merrion Square, Dublin 2, Ireland. [12]Landessternwarte, Königstuhl, D-69117 Heidelberg, Germany. [13]Laboratoire d'Astrophysique de Grenoble, INSU/CNRS, Université Joseph Fourier, BP 53, F-38041 Grenoble Cedex 9, France. [14]DAPNIA/DSM/CEA, CE Saclay, F-91191 Gif-sur-Yvette, France. [15]Unit for Space Physics, North-West University, Potchefstroom 2520, South Africa. [16]Laboratoire de Physique Nucléaire et de Hautes Energies, IN2P3/CNRS, Universités Paris VI & VII, 4 Place Jussieu, F-75252 Paris Cedex 05, France. [17]Institut für Theoretische Physik, Lehrstuhl IV: Weltraum und Astrophysik, Ruhr-Universität Bochum, D-44780 Bochum, Germany. [18]Institute of Particle and Nuclear Physics (IPNP), Charles University, V Holesovickach 2, 180 00 Prague 8, Czech Republic. [19]University of Namibia, Private Bag 13301, Windhoek, Namibia.

*Present address: Université Libre de Bruxelles, Faculté des Sciences, Campus de la Plaine, CP230, Boulevard du Triomphe, 1050 Bruxelles, Belgium.
†To whom correspondence should be addressed. E-mail: denauroi@in2p3.fr; dubus@in2p3.fr

with photon indices Γ close to 2, which suggests that emission could extend to the >100 GeV regime where atmospheric Cherenkov telescope (ACT) arrays operate. Constraining the emission cutoff energy provides important clues as to the physics of the γ-ray source. Furthermore, the XRB associations can be rigorously tested by the superior angular resolution of ACTs.

Located in the Southern Hemisphere, LS 5039 is ideally accessible to the High Energy Stereoscopic System (HESS). HESS is an ACT array of four telescopes located in Namibia, each equipped with a 107-m^2 mirror and a 960–photomultiplier tube camera (*12*–*14*). The telescopes image the Cherenkov light from showers of particles created when VHE γ-rays and cosmic rays enter the atmosphere. A central trigger selects only showers seen by at least two telescopes. The combination of high-resolution imaging and stereoscopic shower reconstruction allows efficient rejection of the background from cosmic ray–initiated showers. The HESS 5σ sensitivity above 100 GeV reaches 1% of the Crab Nebula flux after 25 hours of observations close to the zenith. The direction of each γ-ray shower is determined to 0.1° accuracy, which enables localization of sources in the arc min range within the 5° field of view.

The galactic plane survey carried out by the full HESS array in the summer of 2004 testifies to the performance of the instrument. In (*15*) we reported on the discovery of eight extended VHE γ-ray sources within ±30° of the galactic center and ±3° of the plane. After standard quality selection, a total of 25 pointings (10.5 hours live time) taken during the scan were found to cover the position of LS 5039. The data were independently analyzed with two separate calibration pipelines (*16*) and several different reconstruction methods, all of which were in excellent agreement with each other. The results presented here are based on a maximum likelihood adjustment of a shower model to the observed images to obtain the direction, impact parameter, and energy of the primary (*17*). The likelihood adjustment also provides for each event a probability that is used to select the γ-ray–like events. An image size cut of 60 photoelectrons was applied to avoid systematic effects due to inhomogeneities of the night sky background over the field of view, corresponding to an average postcut spectroscopic energy threshold of 220 GeV.

The reconstructed γ-ray map shows an excess 1° southwest of the previously reported hotspot HESS J1825-137 (Fig. 1). The excess on each position is calculated by comparing the number of source events, integrated over the instrument point spread function, against the estimated number of background events in the same region.

We find a significance of more than 7σ for this new source, denoted HESS J1826-148. The source is point-like with a size upper limit of 50 arc sec (1σ) given by a likelihood fit to a Gaussian source profile folded through the detector response. This is actually the only point-like source discovered in the galactic scan (*15*). The best position is α(J2000) = $18^h26^m15^s$ and δ = –14°49′30″ (with statistical and systematic uncertainties of ±32 and ±30 arc sec, respectively, comparable to the uncertainties of the other survey sources) (*15*). The positional accuracy is limited by the presence of an extended nearby source and systematics in observations taken at large offsets. On-axis observations should improve the positional error to better than 15 arc sec.

The γ-ray spectrum was derived from the comparison of reconstructed event energies (in a circle of 6 arc min around the source) to the prediction for a given spectral shape (*18*). The prediction uses energy resolutions and system acceptances derived from simulations, taking into account the zenith angle pointing of the array and the off-axis angle of the shower in the field of view for each observation. We find an acceptable fit (chance probability of 7% of getting a worse fit) to a power law with a photon index Γ = 2.12 ± 0.15 (Fig. 2). The low statistics currently limit further investigation of more complex spectral shapes. The average integral flux above 250 GeV is 5.1 × 10^{-12} photons cm^{-2} s^{-1} (with statistical and systematic uncertainties of ±0.8 and ±1.3 photons cm^{-2} s^{-1}, respectively), corresponding to a luminosity of ~10^{33} erg s^{-1} at 3 kpc (*11*). Errors on the spectral parameters correspond to 1σ confidence intervals. The highest energy measurement is at ~4 TeV.

The positions of the supernova remnant G16.8-1.1 and the pulsar PSR B1822-14, which are both in the error box of the EGRET source and are both plausible γ-ray sources, are inconsistent with the position of HESS J1826-148 (Fig. 1). Production of γ-rays from the interaction of cosmic rays with the interstellar medium is precluded by the low H column density at the location of HESS J1826-148 relative to its surroundings (*19*). The radio position of LS 5039 is 84 arc sec away from the HESS position and well within the 3σ confidence region (Fig. 1). We verified that there are no other radio or x-ray sources compatible with HESS J1826-148 in the National Radio Astronomy Observatory (NRAO) Very Large Array 1.4-GHz Sky Survey (*20*) and in the XMM/Chandra fields analyzed by (*21*). The observations are not simultaneous

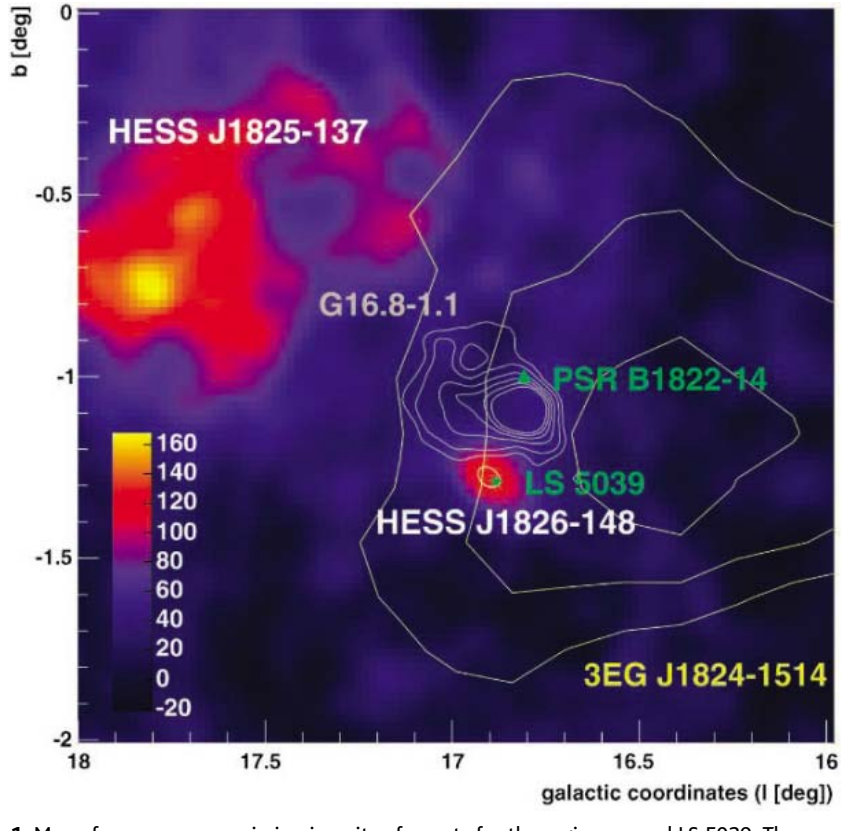


Fig. 1. Map of excess γ-ray emission in units of counts for the region around LS 5039. The map has been smoothed by the point spread function. The white ellipse shows the 3σ confidence region for HESS J1826-148. The radio emission from the SNR G16.8-1.1 is represented by gray contours (0.05, 0.1, 0.2, 0.3, 0.4, and 0.5 Jy/beam) obtained from the Parkes-MIT-NRAO 6-cm radio survey map (*27*, *28*). The yellow contours show the 68%, 95%, and 99% confidence level regions of the EGRET source 3EG J1824-1514. The green star marks the position of the radio source associated with LS 5039. HESS J1825-137 is discussed in (*15*).

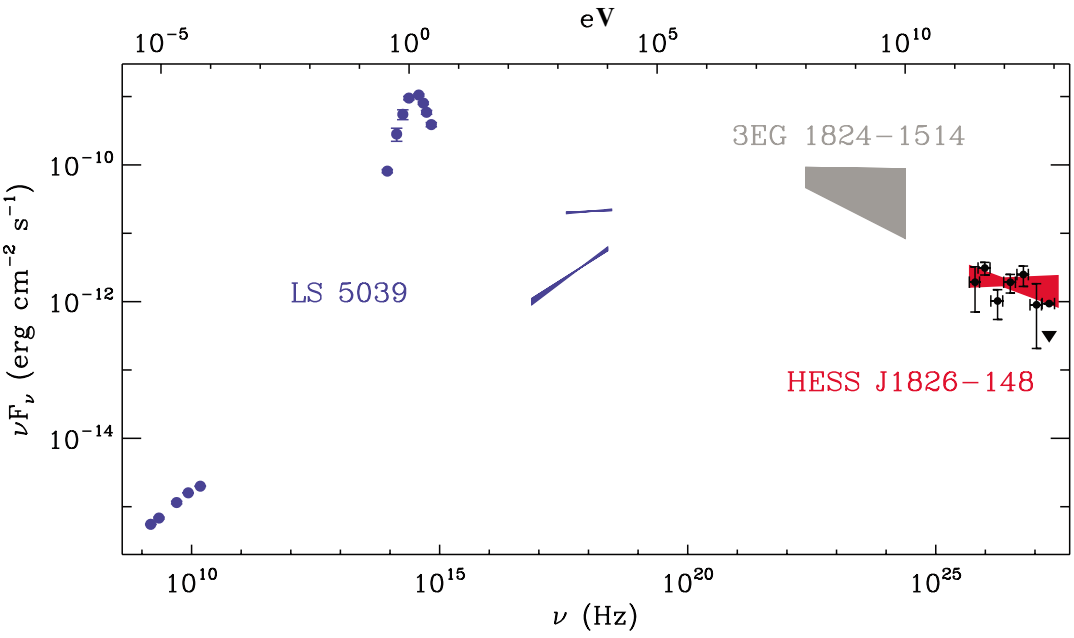


Fig. 2. Spectral energy distribution of LS 5039 including the spectrum of HESS J1826-148 [points and upper limit (inverted triangle) in black, power-law fit in red]. The average radio, optical, and x-ray fluxes are shown in blue (*21*, *28*–*30*). Optical fluxes are not dereddened. The two blue x-ray spectra correspond to the historical 1998 high (Rossi X-ray Timing Explorer, RXTE) and 2003 low (X-ray Multi-mirror Mission, XMM) flux observations of LS 5039. The multiyear average flux above 100 MeV from the EGRET source 3EG J1824-1514 is shown in gray (*9*). The *y* axis is in units of flux times frequency ("spectral luminosity").

with those of HESS, so we cannot formally exclude a long episode of flaring from a blazar. It would be surprising if this blazar were not detected in radio, as blazar emission at this wavelength is persistent at levels of ~100 mJy, well above the survey sensitivity (3 mJy for a point source). The present evidence largely favors the association of LS 5039 with HESS J1826-148 and, by extrapolation, with the unidentified EGRET source 3EG J1824-1514. The present HESS data are consistent with a constant flux (fig. S1). Confirmed γ-ray variability correlated with other wavebands or a telltale modulation would fully establish the association.

Several processes can lead to γ-ray emission in LS 5039. The bulk of the luminosity in the system is emitted by the O6.5V stellar companion ($L_* \approx 10^{39}$ erg s^{-1}) at an energy $kT_* \approx$ 3.5 eV (Fig. 2). The binary separation varies from $2R_*$ to $6R_*$ (where $R_* \approx 7 \times 10^{11}$ cm), and the radiation density reaches $n_* \approx 10^{14}$ photons cm^{-3} close to the compact object. These stellar photons can be boosted to γ-ray energies by inverse Compton scattering on VHE electrons (*22*). With such radiation densities, the energy loss time scale for electrons in the deep Klein-Nishina regime, giving a strict upper limit on the radiative time scale, is ~300 s. A short radiative time scale relative to the escape time scale from the system (~100 s) implies that inverse Compton emission can be very efficient.

Accelerating electrons to the required energies may be hindered by such rapid losses. Very high energies may be easier to reach for protons, which suffer fewer radiation losses. VHE γ-rays may then be emitted via proton-proton interactions with the stellar wind. Assuming a stellar wind with a mass loss rate of 10^{-6} solar masses per year and a velocity of 1000 km s^{-1}, the density at $2R_*$ is $\sim 10^{10}$ protons cm^{-3}. For such a density, the proton-proton interaction time scale is $\sim 10^5$ s. At least $10^2/10^5 = 0.1\%$ of the protons radiate for free-streaming particles, implying a total kinetic energy of less than 10^{38} erg s^{-1}. Protons may also interact with stellar photons, but the threshold is very high ($\sim 10^{17}$ eV) and the corresponding interaction time scale is $\sim 10^3$ s.

Photons emitted in the HESS energy range can interact with the 3.5-eV stellar radiation before leaving the system, producing e^+e^- pairs. The cross-section maximum $\sigma_{\gamma\gamma} \approx 1.7 \times 10^{-25}$ cm^2 occurs for γ-rays of energy $\approx$ 100 GeV. The opacity is $\tau_{\gamma\gamma} = \sigma_{\gamma\gamma} n_* r \approx 20$ for a photon traveling a distance $r \approx 10^{12}$ cm (comparable to the binary separation). VHE photons emitted close to the compact object are therefore always well inside the "γ-photosphere" at which $\tau_{\gamma\gamma} \approx 1$. This initiates an e^+e^- pair cascade that redistributes the absorbed radiation to lower frequencies. Gamma rays at energies below 100 GeV suffer little absorption because of the Wien cutoff of the stellar spectrum. At higher energies, the opacity decreases as $1/E_\gamma$ (*23*). The VHE spectrum may therefore be hardened relative to its intrinsic shape.

The absorption of TeV photons in the system can be mitigated. First, the cross-section threshold and amplitude are angle-dependent, so that γ-rays emitted in a cone pointing away from the companion are not absorbed. Scattering of stellar photons in the wind will tend to isotropize the radiation and diminish this effect. Variations are expected because the geometry changes with orbital phase. Second, γ-ray emission need not take place close to the compact object. Observations of x-ray emission from XRB jets provide evidence for acceleration of electrons to TeV energies on parsec scales (*24*). In LS 5039, acceleration at a shock >1 AU away from the stellar companion would happen beyond the γ-photosphere.

The association of LS 5039 with HESS J1826-148 confirms that, like some AGN, XRBs are able to accelerate particles to at least TeV energies. Shocks from colliding ejecta or from jet–interstellar medium interactions are natural candidates. Yet the association with an outflow may be questioned in the absence of a direct detection of relativistic motion in radio. The relativistic wind of a young pulsar is a conceivable alternative for particle injection (*25*). The situation would then resemble that in PSR B1259-63, a system composed of a radio pulsar in a much wider 3.4-year eccentric orbit around a Be star. TeV emission from PSR B1259-63 was detected with HESS close to periastron (*26*). The higher wind density in LS 5039 probably smears out any radio pulses. The resolved radio emission from LS 5039 would be due to particles (cascade pairs) streaming out of the system. Further insights into this system may be gained from combined radio and γ-ray observations: Very Long Baseline Array maps attain a spatial resolution of a few AU (*6*) tantalizingly close to the γ-photosphere.

References and Notes

1. I. F. Mirabel, L. F. Rodriguez, *Nature* **371**, 46 (1994).
2. A. P. Marscher *et al.*, *Nature* **417**, 625 (2002).
3. T. C. Weekes, *Space Sci. Rev.* **59**, 315 (1992).
4. P. C. Gregory, A. R. Taylor, *Nature* **272**, 704 (1978).
5. A. R. Taylor, H. T. Kenny, R. E. Spencer, A. Tzioumis, *Astrophys. J.* **395**, 268 (1992).
6. J. M. Paredes, J. Martí, M. Ribó, M. Massi, *Science* **288**, 2340 (2000).
7. J. B. Hutchings, D. Crampton, *Publ. Astron. Soc. Pac.* **93**, 486 (1981).
8. M. V. McSwain *et al.*, *Astrophys. J.* **600**, 927 (2004).
9. R. C. Hartman *et al.*, *Astrophys. J. Suppl. Ser.* **123**, 79 (1999).

10. G. F. Bignami, P. A. Caraveo, R. C. Lamb, T. H. Markert, J. A. Paul, *Astrophys. J.* **247**, L85 (1981).
11. C. Motch, F. Haberl, K. Dennerl, M. Pakull, E. Janot-Pacheco, *Astron. Astrophys.* **323**, 853 (1997).
12. K. Bernlöhr *et al.*, *Astropart. Phys.* **20**, 111 (2003).
13. J. A. Hinton, *N. Astron. Rev.* **48**, 331 (2004).
14. S. Funk *et al.*, *Astropart. Phys.* **22**, 285 (2004).
15. F. Aharonian *et al.*, *Science* **307**, 1938 (2005).
16. F. Aharonian *et al.*, *Astropart. Phys.* **22**, 109 (2004).
17. M. de Naurois *et al.*, in *28th International Cosmic Ray Conference*, T. Kajita *et al.*, Eds. (Universal Academy Press, Tokyo, 2003), p. 2907.
18. F. Piron *et al.*, *Astron. Astrophys.* **374**, 895 (2001).
19. M. Ribó *et al.*, *Astron. Astrophys.* **384**, 954 (2002).
20. J. J. Condon *et al.*, *Astron. J.* **115**, 1693 (1998).
21. A. Martocchia, C. Motch, I. Negueruela, *Astron. Astrophys.* **430**, 245 (2005).
22. V. Bosch-Ramon, J. M. Paredes, *Astron. Astrophys.* **417**, 1075 (2004).
23. R. J. Gould, G. P. Schréder, *Phys. Rev.* **155**, 1408 (1967).
24. S. Corbel *et al.*, *Science* **298**, 196 (2002).
25. L. Maraschi, A. Treves, *Mon. Not. R. Astron. Soc.* **194**, 1P (1981).
26. M. Beilicke, M. Ouchrif, G. Rowell, S. Schlenker, *IAU Circ. 8300*, 2 (2004).
27. N. J. Tasker, J. J. Condon, A. E. Wright, M. R. Griffith, *Astron. J.* **107**, 2115 (1994).
28. M. Ribó, P. Reig, J. Martí, J. M. Paredes, *Astron. Astrophys.* **347**, 518 (1999).
29. J. Martí, J. M. Paredes, M. Ribó, *Astron. Astrophys.* **338**, L71 (1998).
30. J. S. Clark *et al.*, *Astron. Astrophys.* **376**, 476 (2001).
31. The support of the Namibian authorities and of the University of Namibia in facilitating the construction and operation of HESS is gratefully acknowledged, as is the support of the German Ministry for Education and Research (BMBF), the Max Planck Society, the French Ministry for Research, the CNRS-IN2P3 and the Astroparticle Interdisciplinary Programme of the CNRS, the UK Particle Physics and Astronomy Research Council (PPARC), the IPNP of Charles University, the South African Department of Science and Technology and National Research Foundation, and the University of Namibia. We appreciate the excellent work of the technical support staff in Berlin, Durham, Hamburg, Heidelberg, Palaiseau, Paris, Saclay, and Namibia in the construction and operation of the equipment. L.C., C.M., M.O., and M.T. are also affiliated with the European Associated Laboratory for Gamma-Ray Astronomy, jointly supported by CNRS and the Max Planck Society.

Supporting Online Material
www.sciencemag.org/cgi/content/full/1113764/DC1
Fig. S1
References

19 April 2005; accepted 16 June 2005
Published online 7 July 2005;
10.1126/science.1113764
Include this information when citing this paper.

Spectroscopy Using Quantum Logic

P. O. Schmidt,*† T. Rosenband, C. Langer, W. M. Itano, J. C. Bergquist, D. J. Wineland

We present a general technique for precision spectroscopy of atoms that lack suitable transitions for efficient laser cooling, internal state preparation, and detection. In our implementation with trapped atomic ions, an auxiliary "logic" ion provides sympathetic laser cooling, state initialization, and detection for a simultaneously trapped "spectroscopy" ion. Detection is achieved by applying a mapping operation to each ion, which results in a coherent transfer of the spectroscopy ion's internal state onto the logic ion, where it is then measured with high efficiency. Experimental realization, by using $^{9}Be^{+}$ as the logic ion and $^{27}Al^{+}$ as the spectroscopy ion, indicates the feasibility of applying this technique to make accurate optical clocks based on single ions.

The continued development of atomic-state manipulation techniques has led to improvements in physical measurements and devices. A good example of this improvement is in the area of atomic spectroscopy, where increases in resolution and accuracy to levels better than 1 part in 10^{15} (*1*, *2*) have been achieved with atomic clocks. This, in turn, has improved tests of fundamental theories (*3*). To achieve these levels of performance in spectroscopy, four key requirements are as follows: (i) selection of an atom with a good reference or "spectroscopy" transition, which is suitably narrow and relatively immune to environmental perturbations; (ii) cooling to minimize velocity-induced frequency shifts; (iii) reliable initial state preparation; and (iv) efficient state detection.

Previously, to satisfy these requirements, an atom was chosen that simultaneously had a good spectroscopy transition and other, more strongly allowed transitions to accomplish requirements (ii) to (iv) (*1*, *2*). Here we describe how two different atomic species can share these requirements, so that one species need only have a good spectroscopy transition, and the other species can fulfill the other three requirements, as outlined in (*4*). We demonstrate this technique experimentally with trapped atomic ions.

To describe the transfer protocol, we consider two atomic ions of different species located on the weak axis of a three-dimensional trap. We designate one of the ions as the logic ion and the other as the spectroscopy ion, and we treat the internal states of the ions as two-level systems with eigenstates labeled $|\downarrow\rangle$ and $|\uparrow\rangle$. The Coulomb interaction of the ions couples their motion, which is best described in a normal-mode basis (*5*–*7*). All normal modes are assumed to be initially cooled to near their ground states by Doppler laser cooling on the logic ion, which sympathetically cools the spectroscopy ion (*8*).

One of the ions' normal modes (which has harmonic oscillator states labeled $|n\rangle_m$, where n denotes the nth level of the mode m) is chosen as the transfer mode on which the state mapping is implemented. This mode is cooled to its ground state (*7*). Figure 1A shows both ions and the transfer mode in their ground states, described by the wave function $\psi_0 = |\downarrow\rangle_S|\downarrow\rangle_L|0\rangle_m$, where the indices S and L denote the spectroscopy and logic ion, respectively. We excite the spectroscopy transition by applying coherent radiation tuned near the spectroscopy ion's transition resonance (Fig. 1B), leading to

$$\Psi_0 \rightarrow \Psi_1 = (\alpha|\downarrow\rangle_S + \beta|\uparrow\rangle_S)|\downarrow\rangle_L|0\rangle_m$$
$$= (\alpha|\downarrow\rangle_S|0\rangle_m + \beta|\uparrow\rangle_S|0\rangle_m)|\downarrow\rangle_L \quad (1)$$

where $|\alpha|^2 + |\beta|^2 = 1$. We now drive a red sideband (RSB) π pulse (*5*, *9*) on the spectroscopy ion (Fig. 1C) so that

$$\Psi_1 \rightarrow \Psi_2 = (\alpha|\downarrow\rangle_S|0\rangle_m + \beta|\downarrow\rangle_S|1\rangle_m)|\downarrow\rangle_L$$
$$= |\downarrow\rangle_S|\downarrow\rangle_L(\alpha|0\rangle_m + \beta|1\rangle_m) \quad (2)$$

thereby mapping the spectroscopy ion's internal state to the transfer mode, which is shared by both ions. During the mapping process, a transient entanglement is created between the internal state of the $^{27}Al^{+}$ ion and the motional state. The $|\downarrow\rangle_S|0\rangle_m$ component of the wave function is unaffected by this operation, because the state $|\uparrow\rangle_S|-1\rangle_m$ does not exist (*5*, *9*); this is the key element of quantum logic used here (*10*, *11*). We apply a final RSB π pulse on the logic ion (Fig. 1D), which yields

$$\Psi_2 \rightarrow \Psi_{\text{final}} = |\downarrow\rangle_S(\alpha|\downarrow\rangle_L + \beta|\uparrow\rangle_L)|0\rangle_m \quad (3)$$

and thereby completes the mapping of the spectroscopy ion's state onto the logic ion.

The logic ion is next measured (not shown in Fig. 1), projecting its state to $|\downarrow\rangle_L$ or $|\uparrow\rangle_L$, which can be efficiently distinguished. By repeating this experiment many times, we can determine the probabilities $|\alpha|^2$ and $|\beta|^2$ as functions of the spectroscopy probe frequency and thereby determine the spectroscopy ion's transition frequency.

We implemented the technique with a single $^{9}Be^{+}$ logic ion (*5*, *11*) and a single $^{27}Al^{+}$ spectroscopy ion (Fig. 2) simultaneously trapped in a linear Paul trap similar to that de-

Time and Frequency Division, National Institute of Standards and Technology, 325 Broadway, Boulder, CO 80305, USA.

*Present address: Institut für Experimentalphysik, Universität Innsbruck, Technikerstrasse 25, A-6020 Innsbruck, Austria.
†To whom correspondence should be addressed. E-mail: piet.schmidt@uibk.ac.at

scribed in (*12*). Our choice for $^{27}Al^+$ was motivated by its potential as a high-accuracy optical clock based on the $^1S_0 \leftrightarrow {}^3P_0$ reference transition, which has a Q factor of $Q \equiv f/\Delta f \approx 2 \times 10^{17}$, where f is the frequency of the transition and Δf is its width (*13*). Although it was previously considered (*14*, *15*), $^{27}Al^+$ has not yet been used as an optical frequency standard because it lacks an accessible cooling transition.

As a demonstration of the technique, we probed the magnetic substates of the $|{}^1S_0, F = 5/2\rangle \rightarrow |{}^3P_1, F' = 7/2\rangle$ transitions, where F and F' are the total angular momentum quantum numbers of the ground and excited state, respectively, in $^{27}Al^+$ [wavelength $\lambda \approx 267$ nm, natural linewidth $\Gamma/2\pi \approx 520$ Hz (*16*)]. We drove these transitions with a frequency-doubled dye laser that had been stabilized to an isolated high-finesse cavity (*17*), yielding a spectral linewidth of less than 6 Hz. Two separately switchable probe laser beams, incident at 45° with respect to the weak trap axis, were $\hat{\pi}$- and $\hat{\sigma}^{+/-}$-polarized, respectively.

The $^9Be^+$ system, with $|\downarrow\rangle_L \equiv |{}^2S_{1/2}, F = 2, m_F = -2\rangle$ and $|\uparrow\rangle_L \equiv |{}^2S_{1/2}, F' = 1, m_{F'} = -1\rangle$, where m_F is the magnetic quantum number of the total angular momentum F, has been described in detail (*7*, *11*). The trap provides single $^9Be^+$ trap frequencies of $\omega_z \approx 2\pi \times 3.8$ MHz along the weak axis (z axis) and $\omega_x \approx 2\pi \times 13.8$ MHz and $\omega_y \approx 2\pi \times 14.9$ MHz orthogonal to the weak trap axis. We performed Doppler cooling with a $\hat{\sigma}^-$-polarized laser beam tuned to the $^9Be^+$ $|\downarrow\rangle_L \rightarrow |{}^2P_{3/2}, F'' = 3, m_{F''} = -3\rangle$ transition. Counting fluorescence photons on this cycling transition also allowed us to determine the $^9Be^+$ ion's final measured state, because an ion in $|\downarrow\rangle_L$ fluoresces strongly, whereas an ion in $|\uparrow\rangle_L$ fluoresces negligibly for this laser frequency (*11*, *14*). State rotations and resolved-sideband cooling on $^9Be^+$ were performed by means of two-photon stimulated Raman pulses on $|\downarrow\rangle_L|n\rangle_m \leftrightarrow |\uparrow\rangle_L|n'\rangle_m$ transitions (*5*, *11*).

Raman sideband cooling of the two axial modes, optical pumping of $^9Be^+$ (*7*, *11*), and state preparation of the spectroscopy ion (see below) initialize the ion pair to the state $|\downarrow\rangle_S|\downarrow\rangle_L|0\rangle_m$, which is the starting point for the spectroscopy protocol described above. State transfer was carried out using the in-phase weak-axis mode (*7*).

Figure 3A shows experimental data for single–laser pulse (Rabi) spectroscopy of the $^{27}Al^+$ $|{}^1S_0, F = 5/2, m_F = 5/2\rangle = |\downarrow\rangle_S \rightarrow |{}^3P_1, F' = 7/2, m_{F'} = 7/2\rangle = |\uparrow\rangle_S$ transition. We applied interrogation pulses of constant intensity and duration, corresponding to approximate π pulses on this transition when the laser beam was tuned to resonance. An experimental sequence took ~1 ms and was repeated 700 times per data point. The observed linewidth of the transition is Fourier-transform–limited with a fitted full width at half maximum linewidth of 63 kHz for an excitation pulse duration of $t_\pi = 12.6$ μs. The observed contrast of 93% (normalized to the contrast of a $^9Be^+$ sideband transition) was limited by the spontaneous decay of the $^{27}Al^+$ $^3P_1$ state during the protocol and by a shot-to-shot variation of the Rabi frequency caused by fluctuations of phonon number in the Doppler-cooled radial modes (Debye-Waller reduction factors) (*18*, *19*).

Figure 3B shows internal state oscillations on the aluminum ion obtained by varying the interrogation duration t_i (on-resonance Rabi flopping) (*5*). The observed coherence time of 118 μs was limited by the ≈305-μs lifetime of the $^3P_1$ excited state, Rabi frequency fluctuations caused by the radial motion Debye-Waller factors, and magnetic field fluctuations.

We verified the coherence of the transfer process by performing a Ramsey experiment in which the first (optical) π/2 pulse created a superposition state in the aluminum ion. This coherence was then mapped to the beryllium

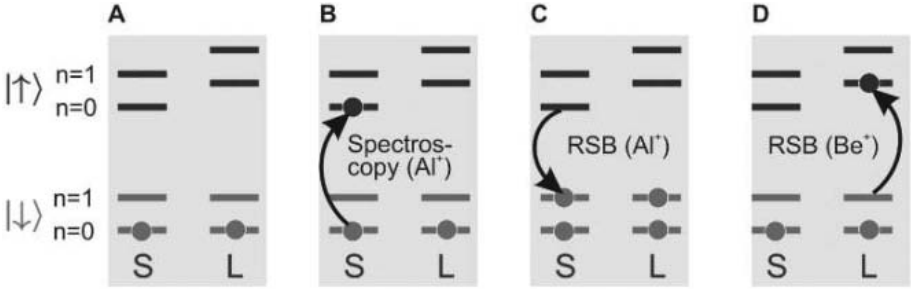


Fig. 1. Spectroscopy and transfer scheme for spectroscopy (S) and logic (L) ions sharing a common normal mode of motion, the transfer mode, with excitation n. (Only the ground and first excited states of the transfer mode are shown.) (**A**) Initialization to the ground internal and transfer-mode states. (**B**) Interrogation of the spectroscopy transition. (**C**) Coherent transfer of the internal superposition state of the spectroscopy ion into a motional superposition state by use of an RSB π pulse on the spectroscopy ion. (**D**) Coherent transfer of the motional superposition state into an internal superposition state of the logic ion by use of an RSB π pulse on the logic ion.

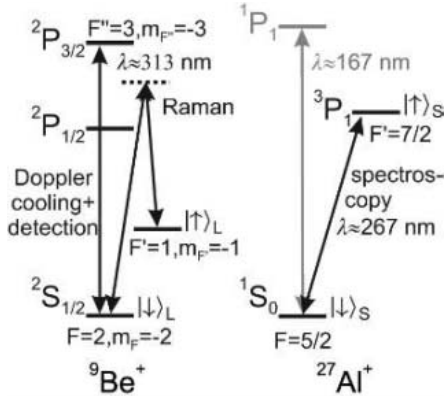


Fig. 2. Partial $^9Be^+$ and $^{27}Al^+$ energy level diagrams (not to scale). Shown are the relevant transitions for Doppler and Raman cooling on the $^9Be^+$ ion, the spectroscopy transition, and the difficult-to-reach Doppler cooling transition at 167 nm on the $^{27}Al^+$ ion.

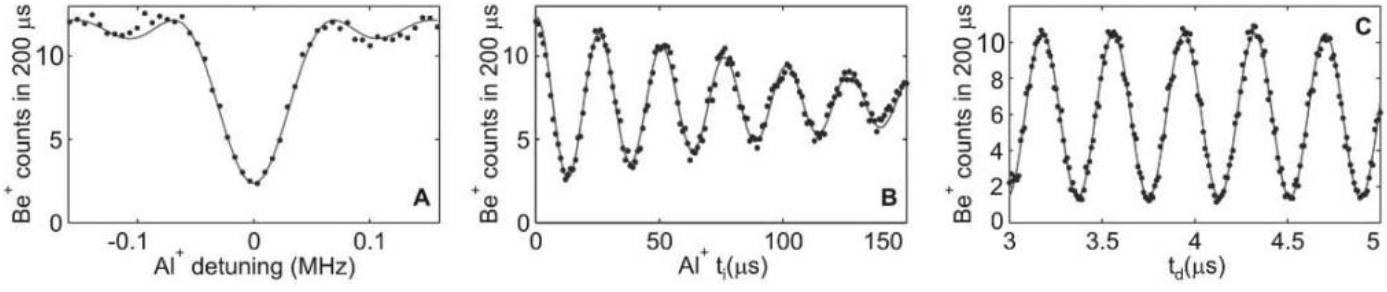


Fig. 3. (**A**) Rabi spectroscopy of the $|{}^1S_0, F = 5/2, m_F = 5/2\rangle \rightarrow |{}^3P_1, F' = 7/2, m_{F'} = 7/2\rangle$ transition in $^{27}Al^+$, showing a frequency scan across the resonance. The data (black circles) are fit by the theoretically expected probability $P_{\downarrow,S}$ of finding $^{27}Al^+$ in the ground state after applying the probe pulse. (**B**) Rabi flopping at the center frequency of the transition in (A). The data are fit by an exponentially damped sinusoidal function. (**C**) Two-ion Ramsey time scan. The data are fit by a sinusoidal function.

ion, where it was probed with a second (Raman transition corresponding to a radio frequency) $\pi/2$ pulse applied to the beryllium ion. Between the two Ramsey pulses, we mapped the aluminum ion's internal state onto the transfer mode, waited a duration t_d, and then mapped the transfer mode state onto the beryllium ion. Figure 3C shows a plot of the detected population $P_{\downarrow,L}$ of the beryllium ion versus t_d. This signal oscillates with a frequency corresponding to the transfer mode frequency. The optical phases of the logic ion and spectroscopy ion laser beams do not need to be stabilized from experiment to experiment, because the phase between the states $|\downarrow\rangle_L$ and $|\uparrow\rangle_L$ after the last Ramsey pulse depends on the phase differences between the two pulses applied to each ion, both of which were held constant over all experiments (*20*).

The spectroscopy protocol does not depend on phase coherence between the laser pulses, because we measure only the spectroscopy ion's state probabilities $|\alpha|^2$ and $|\beta|^2$. However, the coherence of the process can be useful; for example, the Ramsey experiment provides a sensitive way to measure and correct for drifts in the mode frequency. (For this purpose, it is, in practice, easier to perform both Ramsey and mapping pulses on the $^9Be^+$ ion.)

In spectroscopy experiments, initial state preparation is often accomplished with optical pumping on a strongly allowed transition. Because such a transition may not be accessible in the spectroscopy atom, we can again rely on quantum logic methods for state preparation. The process for preparing the $^{27}Al^+$ $|\downarrow\rangle_S \equiv |^1S_0, F = 5/2, m_F = +5/2\rangle$ state is outlined in Fig. 4A. If we assume that the transfer mode is initialized in its ground state, each stage of the preparation process is composed of three steps: (i) a $\hat{\pi}$-polarized, m_F-preserving ($m_{F'} = m_F$) π pulse that does not change the motional state ("carrier" transition); (ii) a $\hat{\sigma}^-$-polarized, m_F-changing ($m_F = m_{F'} + 1$) RSB π pulse; and (iii) a laser cooling stage (on the $^9Be^+$ ion) that puts the transfer mode back into its ground state. The cooling stage incorporates spontaneous emission, which is required in normal optical pumping and makes the RSB π pulse irreversible, hence insuring irreversibility of the overall process. We can "sweep" the population to the desired state $m_F = +5/2$ by sequentially applying these three steps to the $m_F = -5/2, -3/2, \ldots, +3/2$ ground states (in Fig. 4A, only the transfer from the $m_F = +1/2$ and $+3/2$ states is shown). By adapting this procedure, we can prepare any of the $^{27}Al^+$ ground states. In Fig. 4B, we show $\Delta m_F = 0$ spectroscopy on all Zeeman states of the $^{27}Al^+$ $|^1S_0, F = 5/2\rangle \rightarrow |^3P_1, F' = 7/2\rangle$ transition in a magnetic field of 3 mT (chosen to spectrally resolve all transitions) by using this state preparation technique.

We demonstrated in a separate experiment that the effect of optical pumping of the $^{27}Al^+$ during deterministic preparation is negligible. For this purpose, we replaced the RSB pulse in all preparation stages with a wait time of the same duration, thereby allowing for $^{27}Al^+$ $^3P_1 \rightarrow {}^1S_0$ radiative decay during the remaining time (<1.5 ms) of the stage. Figure 4C shows the contrast of the $^{27}Al^+$ $m_F = +5/2 \rightarrow m_{F'} = +5/2$ transition, normalized to the $m_F = +5/2 \rightarrow m_{F'} = +7/2$ transition, as a function of the number of preparation repetitions for both cases. The deterministic preparation achieves the maximum contrast after a single preparation sequence, whereas we observed only a slow increase in contrast for optical pumping (*21*). This result also demonstrates that initial state preparation of spectroscopy ions can be achieved without depending on spontaneous emission from their excited state, which is particularly important for ions with a long-lived excited state, as in the case of the $^{27}Al^+$ $^1S_0 \leftrightarrow {}^3P_0$ clock transition.

We have demonstrated spectroscopy using quantum logic, a technique for precision atomic spectroscopy that removes the requirements of efficient cooling, state preparation, and state detection from the species upon which the spectroscopy is performed. Although we have implemented this technique with atomic ions, it may also be applicable to other systems for which quantum logic techniques are being developed, including neutral atoms (*22*, *23*) and perhaps molecules with simple internal state structure. This technique offers a way to investigate new atomic ion species and their potential for providing reference transitions for high-accuracy optical clocks, such as the $^1S_0 \leftrightarrow {}^3P_0$ transitions in $^{27}Al^+$ and $^{10}B^+$ (*4*, *15*) or optical transitions in He^+ (*24*, *25*). Because coherence of the transfer process is not required for spectroscopy, it should be possible to detect transitions in the spectroscopy ion by detecting the recoil upon absorption (*24*). On the other hand, the coherence in the transfer process could be beneficial in quantum information experiments, where the information could be distributed between different species, thereby simplifying the laser beam addressing of adjacent quantum bits.

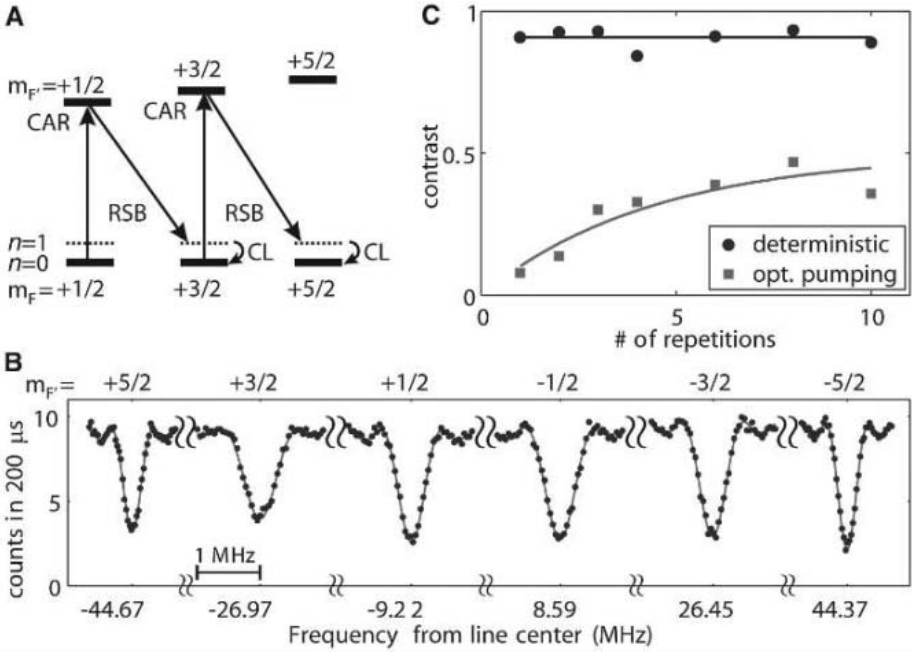


Fig. 4. Deterministic state preparation of the spectroscopy ion. (**A**) The last steps of the deterministic preparation of the $m_F = +5/2$ ground state consist of $\hat{\pi}$-polarized carrier (CAR) π pulses followed by $\hat{\sigma}^-$-polarized RSB π pulses on $^{27}Al^+$ and ground state cooling on $^9Be^+$ (CL), to ensure irreversibility of the sequence. (**B**) Carrier Rabi spectroscopy of all $\hat{\pi}$-polarized transitions in $^{27}Al^+$ using deterministic preparation. (**C**) Normalized contrast of the $m_F = +5/2 \rightarrow +5/2$ $\hat{\pi}$-polarized transition versus number of preparation repetitions for optical pumping and the deterministic preparation. The lines are guides to the eye.

References and Notes

1. P. Gill, Ed., *Proceedings of the 6th Symposium on Frequency Standards and Metrology* (World Scientific, Singapore, 2002).
2. S. A. Diddams, J. C. Bergquist, S. R. Jefferts, C. W. Oates, *Science* **306**, 1318 (2004).
3. S. G. Karshenboim, E. Peik, Eds., *Astrophysics, Clocks and Fundamental Constants*, Lecture Notes in Physics 648 (Springer, Berlin, Heidelberg, 2004).
4. D. J. Wineland, J. C. Bergquist, J. J. Bollinger, R. E. Drullinger, W. M. Itano, in *Proceedings of the 6th Symposium on Frequency Standards and Metrology*, P. Gill, Ed. (World Scientific, Singapore, 2002), pp. 361–368.
5. D. J. Wineland *et al.*, *J. Res. Nat. Inst. Stand. Technol.* **103**, 259 (1998).
6. G. Morigi, H. Walther, *Eur. Phys. J. D* **13**, 261 (2000).
7. M. D. Barrett *et al.*, *Phys. Rev. A.* **68**, 042302 (2003).
8. D. J. Larson, J. C. Bergquist, J. J. Bollinger, W. M. Itano, D. J. Wineland, *Phys. Rev. Lett.* **57**, 70 (1986).
9. D. M. Meekhof, C. Monroe, W. M. Itano, B. E. King, D. J. Wineland, *Phys. Rev. Lett.* **76**, 1796 (1996).

10. J. I. Cirac, P. Zoller, *Phys. Rev. Lett.* **74**, 4091 (1995).
11. C. Monroe, D. M. Meekhof, B. E. King, W. M. Itano, D. J. Wineland, *Phys. Rev. Lett.* **75**, 4714 (1995).
12. M. A. Rowe *et al.*, *Quant. Inform. Comp.* **2**, 257 (2002).
13. W. M. Itano, unpublished calculation.
14. H. G. Dehmelt, *IEEE Trans. Instrum. Meas.* **IM-31**, 83 (1982).
15. N. Yu, H. Dehmelt, W. Nagourney, *Proc. Natl. Acad. Sci. U.S.A.* **89**, 7289 (1992).
16. E. Träbert, A. Wolf, J. Linkemann, X. Tordoir, *J. Phys. B At. Mol. Opt. Phys.* **32**, 537 (1999).
17. B. Young, F. Cruz, W. Itano, J. Bergquist, *Phys. Rev. Lett.* **82**, 3799 (1999).
18. D. J. Wineland, W. M. Itano, *Phys. Rev. A.* **20**, 1521 (1979).
19. For long interrogation times, sideband cooling on the logic ion can be applied during and/or after interrogation of the spectroscopy transition (before the first coherent transfer step, Fig. 1C), thus eliminating a degradation of linewidth and contrast caused by transfer mode heating during interrogation.
20. Methods are available as supporting materials on *Science* Online.
21. We did not prepare the ion in any particular state before each experiment. Rather, competition between state preparation and off-resonant depumping determined the distribution of polarizations before the next repetition of the experiment.
22. K. Eckert *et al.*, *Phys. Rev. A.* **66**, 042317 (2002).
23. E. Charron, E. Tiesinga, F. Mies, C. Williams, *Phys. Rev. Lett.* **88**, 077901 (2002).
24. B. Roth, U. Fröhlich, S. Schiller, *Phys. Rev. Lett.* **94**, 053001 (2005).
25. M. Herrmann *et al.*, in preparation.
26. We thank S. Diddams, J. Koelemeij, and J. Ye for stimulating discussions and comments on the manuscript. This work was supported by the Office of Naval Research, the Advanced Research and Development Activity/National Security Agency, and the National Institute of Standards and Technology (NIST). P.O.S. acknowledges support from the Alexander von Humboldt Foundation. This work is a contribution of NIST, not subject to U.S. copyright.

Supporting Online Material
www.sciencemag.org/cgi/content/full/309/5735/749/DC1
Materials and Methods

3 May 2005; accepted 16 June 2005
10.1126/science.1114375

Electron Localization Determines Defect Formation on Ceria Substrates

Friedrich Esch,[1]* Stefano Fabris,[2] Ling Zhou,[3] Tiziano Montini,[4,5] Cristina Africh,[1,5,6] Paolo Fornasiero,[4,5] Giovanni Comelli,[1,5,6] Renzo Rosei[1,5,6]

The high performance of ceria (CeO_2) as an oxygen buffer and active support for noble metals in catalysis relies on an efficient supply of lattice oxygen at reaction sites governed by oxygen vacancy formation. We used high-resolution scanning tunneling microscopy and density functional calculations to unravel the local structure of surface and subsurface oxygen vacancies on the (111) surface. Electrons left behind by released oxygen localize on cerium ions. Clusters of more than two vacancies exclusively expose these reduced cerium ions, primarily by including subsurface vacancies, which therefore play a crucial role in the process of vacancy cluster formation. These results have implications for our understanding of oxidation processes on reducible rare-earth oxides.

Materials based on ceria (CeO_2) are used in the production and purification of hydrogen, the purification of exhaust gases in three-way automotive catalytic converters, and other catalytic applications (*1*–*4*). In all such applications, highly mobile lattice oxygen is involved in oxidation processes. Over a wide range of working temperatures (from room temperature to 1000°C), ceria plays two key roles: (i) releasing and storing oxygen, and (ii) promoting noble-metal activity and dispersion (*3*, *5*).

Both phenomena are controlled by the type, size, and distribution of oxygen vacancies as the most relevant surface defects. In the case of transition-metal oxide surfaces, a thorough characterization of these vacancies has led to greater understanding of the fundamental features of the reactivity (*6*–*9*) and to the design of efficient supported catalysts (*10*). This knowledge has been lacking for the rare-earth oxides, but their chemistry is likely different because excess electrons left behind by the removal of neutral oxygen localize on empty f states. In ceria, this results in the valence change $Ce^{4+} \rightarrow Ce^{3+}$ of two cations per vacancy and in an extraordinary efficiency for reversible oxygen release (*3*, *11*, *12*). Reduction drastically modifies the reactivity of ceria substrates (*13*, *14*).

Oxygen vacancies are also crucial for the binding of catalytically active species to ceria (*3*). The high activity of Au/ceria catalysts in the water-gas shift reaction has recently been traced back to highly dispersed, ionic Au species that form only in the presence of defects (*15*, *16*).

The control of the density and the nature of oxygen vacancies could provide a means for tailoring the reactivity of ceria-based catalysts. However, despite extensive spectroscopic, microscopic, and diffraction studies, a detailed atomic-level insight into the local defect structure, defect mobility, and valence of the cerium ions is still missing (*3*, *11*, *12*, *14*, *17*–*21*).

Ceria crystallizes in a cubic fluorite structure and exposes the thermodynamically most stable (111) surface (*3*). This surface is the oxygen termination of stoichiometric O-Ce-O trilayers stacked along the [111] direction and also represents the major fraction of the active surface in catalytic nanocrystallites (*12*).

We report high-resolution scanning tunneling microscopy (STM) results on CeO_2(111), which we interpret in light of density functional theory (DFT) calculations. The single-crystal sample was cleaned by repeated cycles of sputtering and annealing to 900°C. Annealing at this temperature leads to oxygen release and is therefore a means to control the degree of surface reduction of the sample. Although ceria is an insulator (band gap 6 eV), its reduction and the elevated temperatures used in this study (300° to 400°C) enhance the electron conductivity (*3*). Furthermore, the elevated temperatures considerably suppress the adsorption of species from the ultrahigh vacuum chamber background (10^{-10} mbar, mainly H_2, H_2O, and CO) that would interfere with the microscope tip. In this way, atomic resolution could be obtained with STM in large-scale images (Fig. 1). The DFT calculations are based on an advanced implementation (*22*–*24*) that captures full electron localization on Ce 4f states in a manner not directly accessible to standard DFT calculations (*25*). Filled-state images are shown to map the positions of the outermost oxygen atoms. Their local relaxation around the defects discriminates between different defect geometries.

Various defects had already been observed in earlier STM and atomic force microscopy studies of ceria at lower temperatures (*17*–*21*), but on a small scale and with limited resolution that precluded a discussion of their relative distribution. Moreover, Namai *et al*. reported defect mobility even at room temperature (*20*, *21*). These results are in contrast to our observations: None of the defects shown in Fig. 1 is mobile on a time scale of minutes. Direct diffusion of vacancies (i.e., hopping of lattice oxygen) on this surface requires temperatures

[1]Laboratorio Nazionale Tecnologie Avanzate e Nanoscienza (TASC)–Istituto Nazionale per la Fisica della Materia (INFM), 34012 Trieste, Italy. [2]Scuola Internazionale Superiore di Studi Avanzati (SISSA) and INFM Democritos National Simulation Center, 34014 Trieste, Italy. [3]Institute of Physical Chemistry and Electrochemistry, University of Hannover, D-30167 Hannover, Germany. [4]Chemistry Department and Consorzio Interuniversitario Nazionale per la Scienza e Tecnologia dei Materiali (INSTM), [5]Center of Excellence for Nanostructured Materials (CENMAT), [6]Physics Department, University of Trieste, 34127 Trieste, Italy.

*To whom correspondence should be addressed. E-mail: friedrich.esch@elettra.trieste.it

higher than 400°C. The low-temperature mobility previously observed (*20*, *21*) can therefore only be rationalized as diffusion of vacancies mediated by adsorbates, or as diffusion of adsorbates interpreted as vacancies.

On the slightly reduced surface (Fig. 1A), single vacancies prevail and can be distinguished as two types (Fig. 2A). One type appears as depressions surrounded by three paired lobes (magenta triangles) and can be assigned to surface O vacancies by comparison to simulated images (Fig. 2C). A second type appears as triple protrusions (cyan triangles) centered around third-layer oxygen sites. By imaging empty states (Fig. 2B), it is clear that the three protrusions belong to a single unit and are not independent entities, such as adsorbates. Simulated STM images identify this type as subsurface O vacancy (Fig. 2C).

STM can therefore precisely detect and measure the single-vacancy distribution. On slightly reduced surfaces, where vacancy clusters (VCs) are not dominant, both surface and subsurface vacancies are present with similar coverages (in Fig. 1A, 1.5% and 1.3% of the surface atoms, respectively) (*26*). This result agrees with our calculations, which predict the same formation energy for the two defects (within 10 meV per vacancy).

The DFT results explain the structural features observed in the STM images. The relaxation of the surface structure around the single vacancies is mainly controlled by the positive electrostatic field centered on the vacancy that repels the nearest neighbor Ce cations and attracts, to a lesser extent, the second nearest neighbor O anions. As a consequence, some of the surface O atoms surrounding the vacancy relax outward. In the case of a single surface vacancy, six atoms relax outward (0.08 to 0.09 Å) and laterally (0.11 to 0.16 Å away from the closest Ce ions) giving rise to the lobes; in the case of a subsurface vacancy, where the field is centered below the surface, only three atoms relax outward (0.19 Å). The same field attracts the two excess electrons near each vacancy, so that the electron localization always occurs on two Ce ions that are nearest neighbors to the defect. The resulting Ce^{3+} ions increase in size and push the neighboring O atoms farther away. This repulsive interaction is centered on the Ce^{3+} ions and therefore breaks the threefold symmetry of the vacancy sites, but this effect is of minor importance, as shown by the simulated STM images (Fig. 2C).

Almost all VCs that form upon further reduction are linear surface oxygen VCs (LSVCs) (92% of the VCs in Fig. 1B). LSVCs appear in three different orientations, reflecting the threefold symmetry of the substrate. Upon closer inspection (Fig. 3A), each LSVC is characterized by a pair of rim O atoms that face each other, one appearing 0.1 Å below (magenta arrow) and one 0.1 Å above the unperturbed surface (cyan arrow). Both atoms are shifted laterally toward the inside of the defect. They constitute a characteristic unit, which we observe once in every LSVC imaged with high resolution. Thus, the double LSVC (upper part of Fig. 3) exhibits mirror symmetry, in contrast to the triple one (lower part).

The simulation of the double LSVC (Fig. 3A, inset) can reproduce the characteristic unit only if an additional subsurface O vacancy is included: The lower O atom has relaxed inward by 0.07 Å, the higher outward by 0.1 Å; both move toward the inside of the defect, in remarkable agreement with the STM results. By contrast, both atoms relax outward (by 0.03 and 0.07 Å) if a double LSVC without subsurface O vacancies is modeled. A double LSVC therefore consists of three oxygen vacancies with the six excess electrons localized on five Ce ions in the first cerium layer and on one Ce ion in the second, so that no Ce^{4+} ions are nearest neighbors to vacancies. Note that this condition cannot be satisfied without the additional subsurface vacancy. Calculations starting from artificial arrangements in which Ce^{3+} ions are placed outside the defect always lead to Ce^{3+} inside the defect.

We conclude that the double LSVC is a complex of a dimer of surface vacancies with one subsurface vacancy, together forming a trimer of vacancies where only Ce^{3+} ions are coordinated to the defect. The exposure of only Ce^{3+} ions also holds for longer LSVCs, where the characteristic unit always appears once and can thus be regarded as the nucleation site that

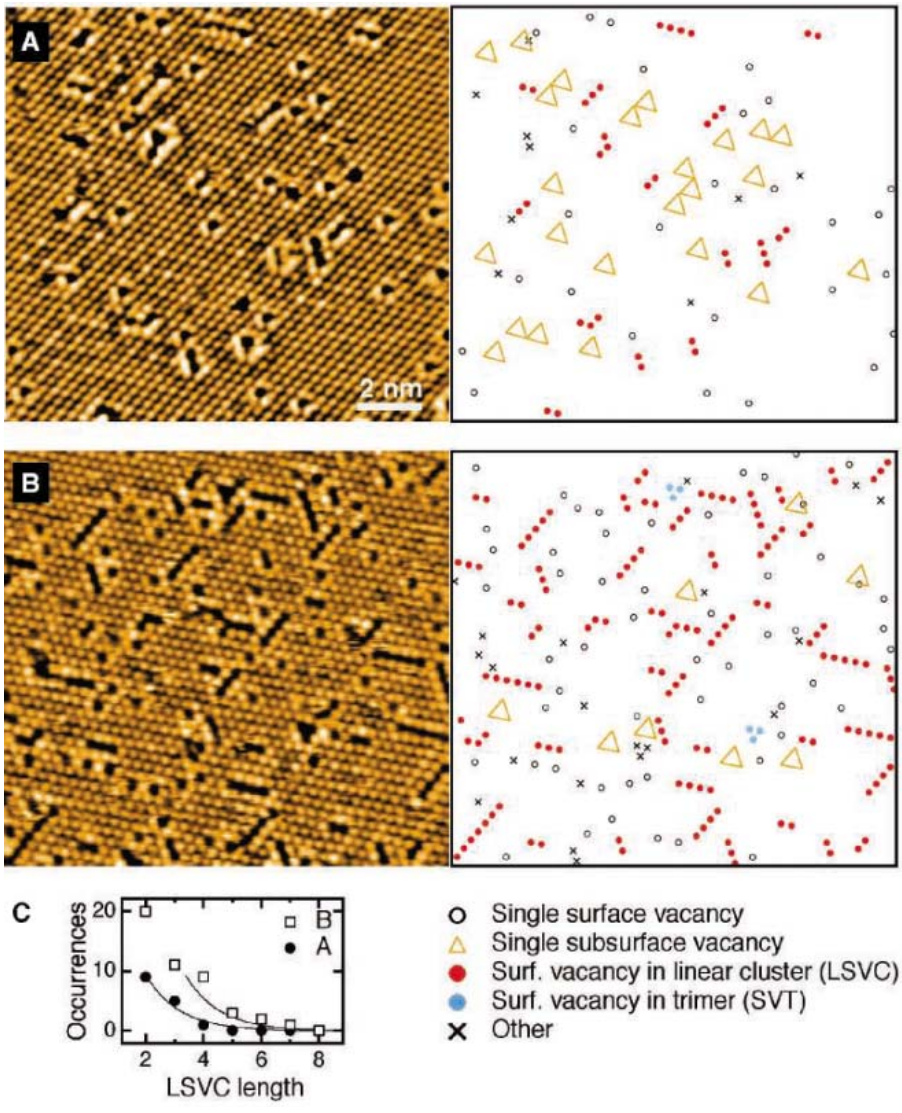


Fig. 1. (**A** and **B**) STM images of the CeO_2(111) surface obtained after 1 min (A) and 5 min (B) of annealing at 900°C, with corresponding representations of the observed defects. (**C**) Histogram of the LSVC distribution as evaluated from (A) (solid circles) and (B) (open squares). The same exponential decay with a rigid shift in defect length can be used to fit both distributions (solid lines). The dominant VCs after prolonged annealing are LSVCs (involving 68% of all O vacancies) and some SVTs (2%); 23% of the O vacancies are single ones. "Other" refers to cases (7%) where the assignment is ambiguous. STM imaging conditions: –3.0 V (sample with respect to tip), 0.3 nA, 300°C.

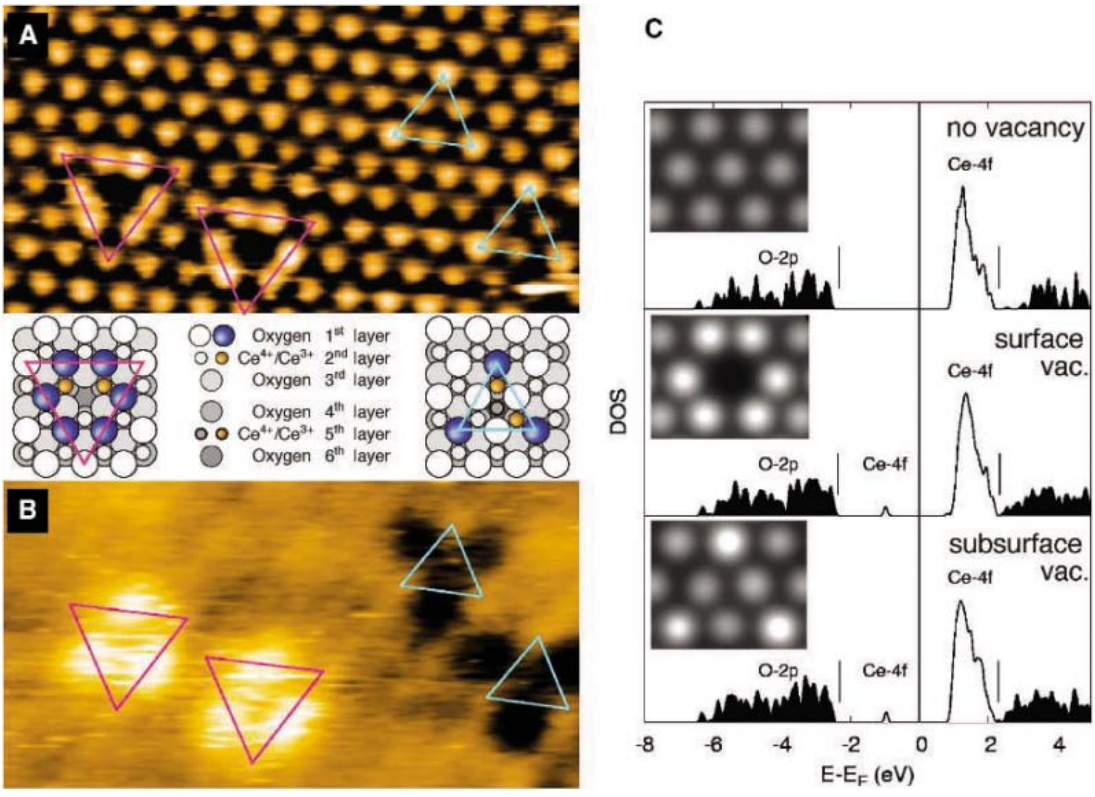


Fig. 2. (**A**) Filled-state and (**B**) empty-state STM images of single vacancies and related structural models (left, surface vacancy; right, subsurface vacancy; characteristic O rim atoms in blue). (**C**) Calculated density of states (DOS) and simulated filled-state STM images (bias –3.0 V). Ce 4f gap states, displayed as unshaded curves, do not contribute to the STM images because of their strong spatial localization. STM imaging conditions: 6.6 nm by 3.5 nm; –3.0 V (A), +3.0 V (B); 0.1 nA.

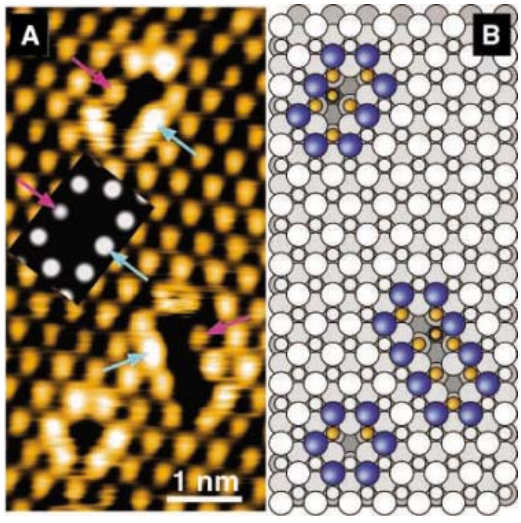


Fig. 3. (**A**) STM image of a double and a triple LSVC as well as a simulated STM image of a double LSVC (inset). The characteristic unit (magenta and cyan arrows) is an indication of the presence of a subsurface vacancy in every LSVC. (**B**) Corresponding structural model. Note the exclusive presence of Ce^{3+} ions in the LSVCs (color code as in Fig. 2). STM imaging conditions: –3.0 V, 0.1 nA.

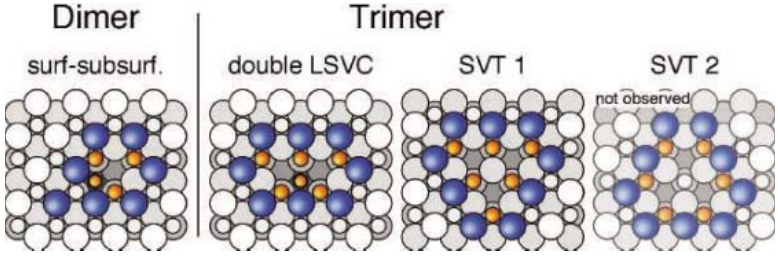


Fig. 4. Structural models of the observed vacancy dimers and trimers (color code as in Fig. 2). The vacancy dimer results from the superposition of the single surface and subsurface vacancies shown in Fig. 2. Removal of a further surface oxygen atom leads to the double LSVC. Of the two possible SVT variants SVT 1 and SVT 2, only the first one, exposing exclusively Ce^{3+} ions, is observed.

triggers defect growth. The removal of an additional terminating O atom results in a longer LSVC, again coordinated exclusively by Ce^{3+} ions. This growth mechanism is independent of the LSVC length (Fig. 1C): Upon further reduction, the length distribution profile is simply shifted to longer defect lengths. The growth of the LSVCs occurs preferentially in a straight manner and without bifurcation (Fig. 1B).

The formation of LSVCs as the dominant oxygen vacancies demonstrates the tendency to form VCs that exclusively expose Ce^{3+} ions. This condition can be fulfilled only for VCs with more than two vacancies; the question arises whether it can be generalized for other VC shapes. The next most abundant case after LSVCs are triangular surface O vacancy trimers (SVTs). Theoretically, two SVT variants are possible, rotated by 60° with respect to each other (Fig. 4): SVT 1 is centered on a subsurface O atom and exposes only six Ce^{3+} ions, whereas SVT 2 is centered on a Ce ion and exposes one Ce^{4+} ion in addition to the six Ce^{3+} ions. Experimentally, all of the observed SVTs have the same orientation (Fig. 1B) (*19*, *20*), which we identify as SVT 1, thus involving only Ce^{3+}. This result confirms that VCs expose only reduced cerium ions.

The decrease of single subsurface vacancies upon prolonged annealing (Fig. 1B with respect to Fig. 1A) is in apparent contradiction to the equal stability of single surface and subsurface vacancies. However, when taking into account all subsurface vacancies (single

and in LSVCs), their fraction increases from 2.2% to 3.0%, similar to the increase in the fraction of single surface vacancies (from 1.5% to 3.0%). This indicates that the subsurface vacancies are essential for LSVC nucleation, which could occur via an intermediate surface–subsurface vacancy dimer (Fig. 4), the only vacancy dimer that has been observed (two occurrences in Fig. 1B, upper right).

Our observations show that electron localization determines which defects are formed on a ceria surface. The structural requirement of one subsurface vacancy per LSVC reveals the high propensity of Ce toward reduction upon O loss: Only Ce^{3+} ions are coordinated to the VC. Although this propensity favors further O release after double LSVCs have been formed, it may hamper their nucleation; the two electrons liberated by each of the first two missing O atoms are insufficient to reduce all of the coordinated Ce ions.

In real catalytic applications, one way to increase oxygen release from ceria while enhancing the thermal stability of its surface area and porosity (*27*) is by doping with Zr^{4+} ions, which are not reduced upon O loss (*28*). These two effects, increased oxygen release and thermal stability, seem irreconcilable but can be rationalized in light of our results: Preliminary calculations show that, with respect to the pure ceria (111) surface, the single vacancy formation around single Zr^{4+} dopants is facilitated by 0.9 eV. Once formed, these vacancies can grow into VCs without further requiring the presence of subsurface vacancies and the related major structural rearrangement.

References and Notes

1. G. A. Deluga, J. R. Salge, L. D. Schmidt, X. E. Verykios, *Science* **303**, 993 (2004).
2. K. Otsuka, T. Ushiyama, I. Yamanaka, *Chem. Lett.* **9**, 1517 (1993).
3. A. Trovarelli, Ed., *Catalysis by Ceria and Related Materials* (Imperial College Press, London, 2002).
4. S. Park, J. M. Vohs, R. J. Gorte, *Nature* **404**, 265 (2000).
5. S. Bernal *et al.*, *Catal. Today* **50**, 175 (1999).
6. R. Schaub *et al.*, *Science* **299**, 377 (2003); published online 12 December 2002 (10.1126/science.1078962).
7. E. Wahlström *et al.*, *Science* **303**, 511 (2004).
8. C. T. Campbell, *Science* **299**, 357 (2003).
9. M. D. Rasmussen, L. M. Molina, B. Hammer, *J. Chem. Phys.* **120**, 988 (2004).
10. M. S. Chen, D. W. Goodman, *Science* **306**, 252 (2004); published online 26 August 2004 (10.1126/science.1102420).
11. D. R. Mullins, S. H. Overbury, D. R. Huntley, *Surf. Sci.* **409**, 307 (1998).
12. L. Wu *et al.*, *Phys. Rev. B* **69**, 125415 (2004).
13. G. R. Rao *et al.*, *J. Catal.* **162**, 1 (1996).
14. A. Martinez-Arias *et al.*, *J. Chem. Soc. Faraday Trans.* **91**, 1679 (1995).
15. Q. Fu, H. Saltsburg, M. Flytzani-Stephanopoulos, *Science* **301**, 935 (2003); published online 3 July 2003 (10.1126/science.1085721).
16. Z.-P. Liu, S. J. Jenkins, D. A. King, *Phys. Rev. Lett.* **94**, 196102 (2005).
17. H. Nörenberg, G. A. D. Briggs, *Phys. Rev. Lett.* **79**, 4222 (1997).
18. H. Nörenberg, G. A. D. Briggs, *Surf. Sci.* **424**, L352 (1999).
19. K. Fukui, Y. Namai, Y. Iwasawa, *Appl. Surf. Sci.* **188**, 252 (2002).
20. Y. Namai, K. Fukui, Y. Iwasawa, *Catal. Today* **85**, 79 (2003).
21. Y. Namai, K. Fukui, Y. Iwasawa, *J. Phys. Chem. B* **107**, 11666 (2003).
22. Strong correlation effects due to localization are modeled by adding a Hubbard-U term to the energy functional expressed in the pseudo-potential plane-wave framework. Details of the calculation can be found in (*23*, *24*) and in the supporting online material.
23. M. Cococcioni, S. de Gironcoli, *Phys. Rev. B* **71**, 035105 (2005).
24. S. Fabris, S. de Gironcoli, S. Baroni, G. Vicario, G. Balducci, *Phys. Rev. B* **71**, 041102 (2005).
25. Z. Yang, T. K. Woo, M. Baudin, K. Hermansson, *J. Chem. Phys.* **120**, 7741 (2004).
26. Statistical evaluation of vacancy occurrences has an error margin of ±0.5% because of some ambiguous defect assignments (see "other" in Fig. 1).
27. G. Colón *et al.*, *Catal. Today* **50**, 271 (1999).
28. P. Fornasiero *et al.*, *J. Catal.* **164**, 173 (1996).

29. We thank M. Gerunda and P. Facci for providing the STM tips, and S. Baroni, S. de Gironcoli, M. Graziani, and F. Rosei for fruitful discussions and critical reading of the manuscript. S.F. thanks Consorzio Interuniversitario CINECA for computing time. Supported by the International Centre for Theoretical Physics (ICTP) Training and Research in Italian Laboratories (TRIL) program (L.Z.), INSTM, Sincrotrone Trieste ScpA, and Ministero dell'Istruzione, dell'Università e della Ricerca (MIUR) under the programs FIRB 2001 (grant RBNE0155X7), COFINLAB 2001 (grant CLAB013P24), and COFIN 2003.

Supporting Online Material

www.sciencemag.org/cgi/content/full/309/5735/752/DC1
Materials and Methods
Fig. S1
References

28 February 2005; accepted 27 May 2005
10.1126/science.1111568

A Light-Actuated Nanovalve Derived from a Channel Protein

Armağan Koçer,[1]* Martin Walko,[2]* Wim Meijberg,[1]† Ben L. Feringa[2]†

Toward the realization of nanoscale device control, we report a molecular valve embedded in a membrane that can be opened by illumination with long-wavelength ultraviolet (366 nanometers) light and then resealed by visible irradiation. The valve consists of a channel protein, the mechanosensitive channel of large conductance (MscL) from *Escherichia coli*, modified by attachment of synthetic compounds that undergo light-induced charge separation to reversibly open and close a 3-nanometer pore. The system is compatible with a classical encapsulation system, the liposome, and external photochemical control over transport through the channel is achieved.

Among addressable nanoscale devices, photoinduced molecular switches stand out for their ability to convert an optical input into a variety of useful output signals (*1*). Their short response times and reversibility allow switching between different states rapidly and repeatedly. These molecular switches can be used to modulate the properties of materials at the bulk level, such as surface wettability (*2*), refractive index (*3*), or the lateral pressure profile of bilayers (*4*), as well as at the single-molecule level, for example, involving host-guest interactions (*5*) or the activity of enzymes (*6*). A particularly challenging and appealing application of photoswitches would be gating channels, thereby externally modulating ion flow and, by extension, drug release. Our approach has been to append an addressable photosensitive gate to a naturally occurring channel protein, which ordinarily controls the exchange of solutes across the lipid bilayers that separate cells and organelles from their environment.

One of the best-characterized channel proteins is the mechanosensitive channel of large conductance, or MscL, from *Escherichia coli* (*7*). In nature, this protein functions as a safety valve to protect the bacterial cell against severe osmotic downshifts (*8*). A sudden influx of water results in the buildup of turgor pressure, generating tension in the membrane. Above a certain threshold value, the pressure results in the opening of a large, nonselective pore in the protein, about 3 nm in diameter (*9*), that allows efflux of ions, small solutes, and even some small proteins in an effort to prevent cell lysis (*10*, *11*). Because of the large energetic cost that this process represents to the cell, the protein is tightly closed under normal conditions. Structural models of the protein and its gating mechanism have their basis in a large body of data from *E. coli* MscL and the crystal structure of its homolog from *Mycobacterium tuberculosis* (*12*–*21*). The protein is a homopentamer with two transmembrane helices, M1 and M2, per subunit. The actual pore of the channel is formed by five M1 helices. Although normally the channel opens in response to tension, the introduction of polar or charged amino acids (*16*) or other charged compounds (*22*) into the 22nd amino acid position of MscL leads to spontaneous opening of the channel; this charge effect also operates in

[1]BiOMaDe Technology Foundation, Nijenborgh 4, 9747 AG Groningen, Netherlands. [2]Department of Organic and Molecular Inorganic Chemistry, Stratingh Institute and Material Science Centre, University of Groningen, Nijenborgh 4, 9747 AG Groningen, Netherlands.

*These authors contributed equally to this work.
†To whom correspondence should be addressed. E-mail: B.L.Feringa@rug.nl (B.L.F.); meijberg@biomade.nl (W.M.)

other gated channels with closed hydrophobic pores (*23*).

In the case of MscL, hydrophilic substitutions into the narrow pore constriction area cause hydration of the pore and weakening of the hydrophobic van der Waals forces responsible for the close packing of the M1 helices in the closed state of the channel. The effect is reinforced if charged or bulky groups are introduced because of electrostatic repulsion and steric factors, respectively. This is reflected in the energetics of the gating transitions, in particular the first subtransition from the closed to the first subconducting state, which is also the major energy barrier for opening the wild-type channel. Within the framework of the "two gates model," this transition represents the opening of the main gate, resulting in an expanded conformation with a low level of conductance attributed to blocking of the pore lumen by a second gate. Opening of this gate needs further energy input and leads to full con-

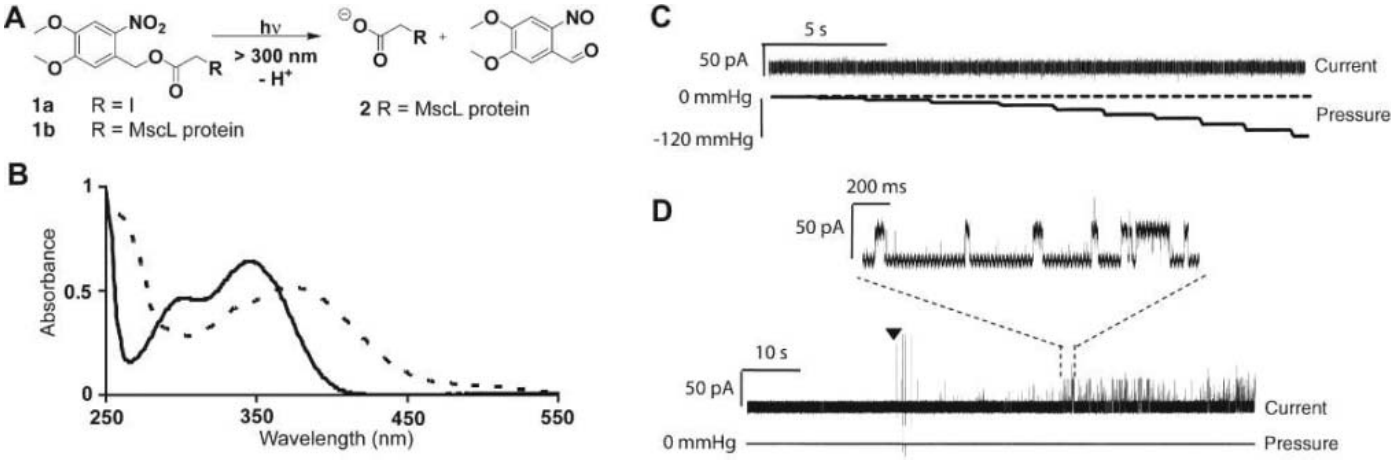


Fig. 1. A one-way photoswitch converts the MscL channel protein into a light-actuated valve. (**A**) Photolysis of compound **1** liberates charged acetate **2**. (**B**) UV-visible spectrum of the solution of **1a** before irradiation (full line) and after 5-min exposure to UV light (>300 nm) (dashed line). The disappearance of the absorption band at 346 nm and appearance of a band at 374 nm indicate the photocleavage of compound **1** into its products. (**C**) The modified MscL channel, which was reconstituted in synthetic lipids {1,2-dioleoyl-sn-glycero-3-phosphocholine, cholesterol, and 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[methoxy(polyethylene glycol)2000] in a molar ratio of 70:20:10, respectively} (*25*), was patched in the dark. Recordings were performed at +20 mV without a pressure gradient in a bath and pipette buffer (200 mM KCl, 100 mM $MgCl_2$, 10 mM $CaCl_2$, 5 mM HEPES, pH = 6.5). Channel openings are shown as upward currents. The light from a high-pressure Hg lamp was directed toward the patch bath through a fiber optic cable, and the wavelength was adjusted by appropriate filters. Current did not flow in the dark even with applied pressure up to the magnitude at which the patch was lost. (**D**) The spontaneous channel openings in response to light were recorded in real time and with no applied pressure. There were no channel openings in the dark (before the triangle); however, upon exposure to light at λ = 366 nm (starting point indicated by a triangle), the channel opened spontaneously. (**Inset**) An expanded fragment of the trace marked by dashed lines.

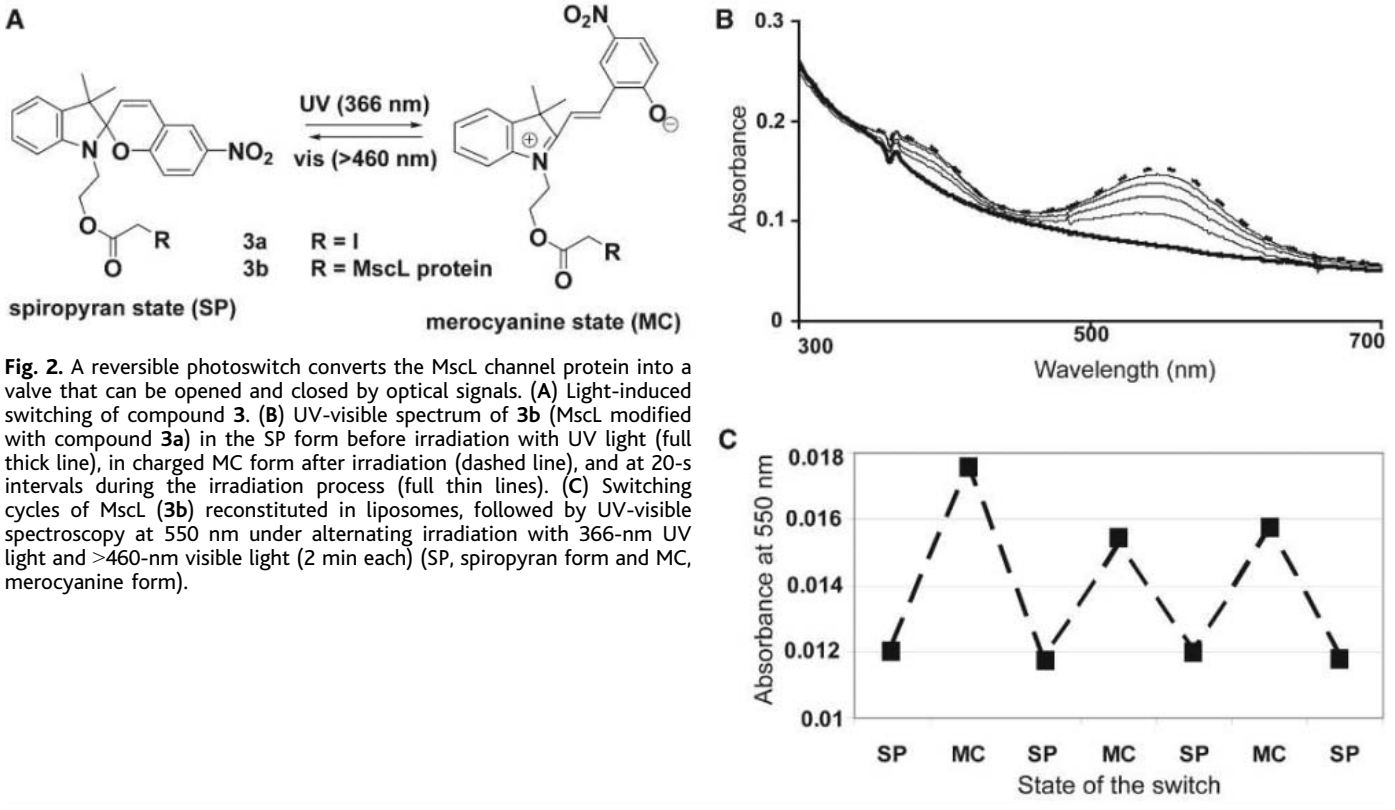


Fig. 2. A reversible photoswitch converts the MscL channel protein into a valve that can be opened and closed by optical signals. (**A**) Light-induced switching of compound **3**. (**B**) UV-visible spectrum of **3b** (MscL modified with compound **3a**) in the SP form before irradiation with UV light (full thick line), in charged MC form after irradiation (dashed line), and at 20-s intervals during the irradiation process (full thin lines). (**C**) Switching cycles of MscL (**3b**) reconstituted in liposomes, followed by UV-visible spectroscopy at 550 nm under alternating irradiation with 366-nm UV light and >460-nm visible light (2 min each) (SP, spiropyran form and MC, merocyanine form).

ductance of the channel [see (*24*) for a detailed discussion].

We prepared several photosensitive compounds specifically to harness the hydration- and charge-mediated gating mechanism described above. In our design, illumination leads to a localized buildup of charge and consequent actuation of the valve. In the case of reversible operation, after initial actuation of the valve illumination with light of another wavelength neutralizes the localized charge and leads to valve closure.

In order to couple such photosensitive actuators specifically to the charge-sensitive part of the channel, we replaced the glycine residue at the 22nd amino acid position in M1 helices by cysteine (*16*), an amino acid that is not present anywhere else in the MscL protein. Five binding sites in each MscL protein were then available for the actuators. Compound **1a**, designed to irreversibly charge the hydrophobic pore of the channel after irradiation, is a cysteine-selective alkylating reagent composed of an iodoacetate bearing the photocleavable protecting group 6-nitroveratryl alcohol (Fig. 1A) (*25*). This compound is sensitive to long-wavelength ultraviolet (UV), a wavelength range that is compatible with most biomaterials (*26*). Illumination at $\lambda > 300$ nm results in photolysis of the protective group, as shown by UV-visible absorption spectroscopy (Fig. 1B), for both free **1a** and the MscL-bound form **1b**. The disappearance of the absorption band at 346 nm and the appearance of the band at 374 nm are typical for the cleavage of esters of 6-nitroveratryl alcohol, producing free acid and 6-nitrosoveratryl aldehyde (*27*). UV photolysis of modified MscL **1b** leaves cysteine-bound acetates **2**, which are negatively charged above pH = 4.0, inside the channel.

The functionality of the resulting protein valve was assessed at the single-molecule level by measuring the ionic current flowing through this modified channel in patch clamp experiments. The modified MscL was reconstituted in synthetic lipids, and patch clamp studies were performed in the presence and absence of UV light in the excised inside-out patch configuration; that is, the inner leaflet of the bilayer formerly facing the liposomal interior becomes exposed to the bath solution. No current flows through the channel in the dark, even if negative pressure is applied up to the breaking point of the patch (Fig. 1C). However, when the proteoliposomes are exposed to UV light, the channel opens even in the absence of applied pressure (Fig. 1D) (*28*).

Having achieved the conversion of the channel protein into a molecular valve actuated by light, we set out to incorporate controlled reversibility. To this end, a photoinduced switch, **3a**, based on a spiropyran core attached to a cysteine-selective iodoacetate moiety was synthesized and coupled to MscL to give **3b** (Fig. 2A). Upon irradiation at 366 nm, photochemical ring opening takes place, resulting in a charged zwitterionic merocyanine structure (MC) as indicated by the appearance of the new absorption maxima at 392 and 552 nm (Fig. 2B). Exposure to visible light (>460 nm) results in the reverse, ring-closing reaction, restoring the original uncharged spiropyran state (SP). This switching cycle can be repeated many times without a noticeable loss of photoactivity of the switch in buffer solution. However, when the switch was attached to the protein, the amount of merocyanine formed on irradiation consistently dropped after the first open-close cycle (Fig. 2C). Because the polarity of the local environment influences the photoequilibrium between the isomers, this result implies a permanent change within the protein after the first UV-visible cycle.

We induced reversible switching between open and closed states of the resulting modified photoactive protein **3b** embedded in a lipid membrane in patch clamp experiments (Fig.

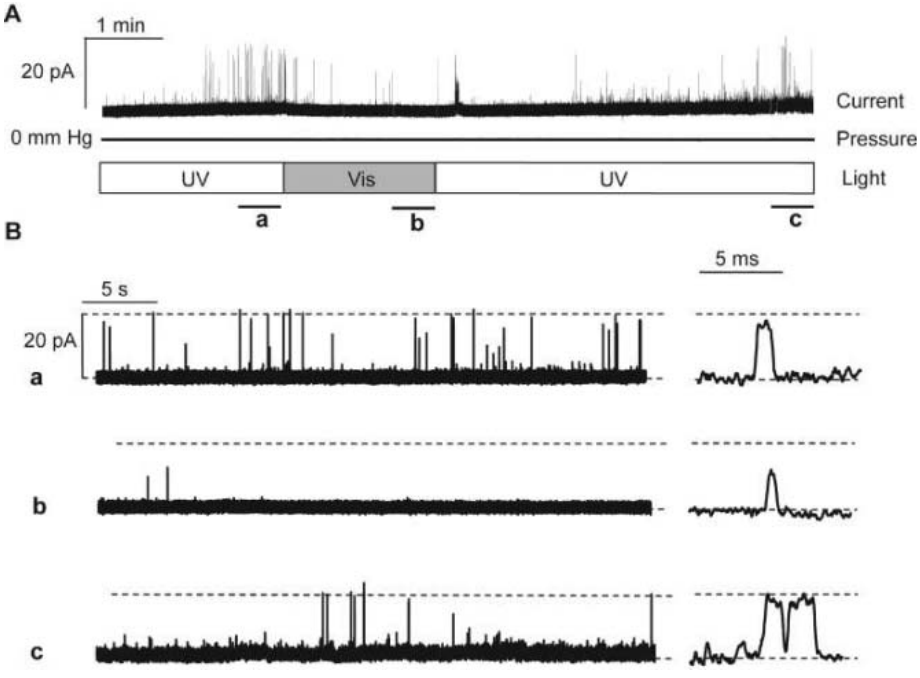


Fig. 3. Electrophysiological analysis of reversible functioning of modified MscL. (**A**) Single-channel recordings were performed at +20 mV without a pressure gradient, and channel openings are shown as upward currents. The patch was sequentially illuminated with UV and visible light by alternating the filter in the light source. Exposure wavelength is indicated under the patch trace. (**B**) Enlarged view of the channel openings at the end of each stimulation. The letters correspond to the positions indicated in the upper trace; (a) and (c) indicate the channel opened by UV, and (b) indicates channel closed by visible light. The first channel of each trace is shown on the right on a magnified time scale.

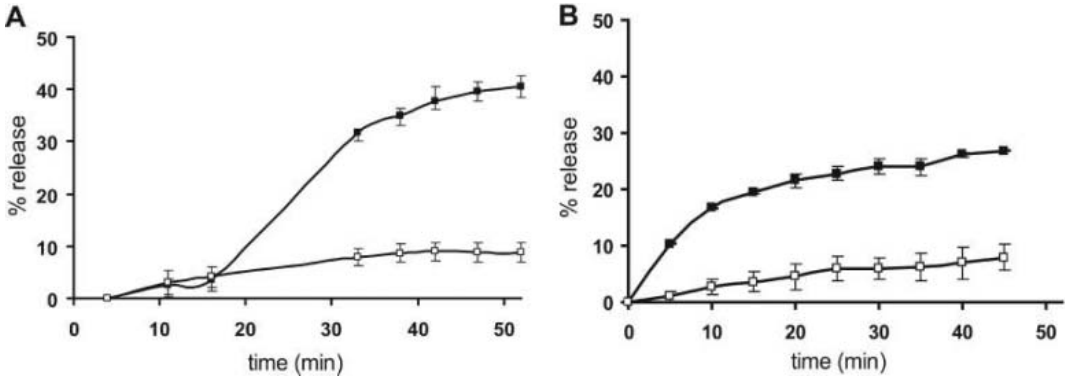


Fig. 4. Functioning of the light-actuated MscL in proteoliposomes. (**A**) Unidirectional operation of modified MscL **1b**. The release of liposomal content was calculated from the relative increase in fluorescence. Open squares indicate the calcein release in the dark, and solid squares indicate MscL-mediated release upon irradiation at 366 nm. (**B**) Reversible operation of modified MscL **3b**. Open squares indicate the fluid release in the dark, and solid squares indicate continuous light-actuated MscL-mediated release. Bars indicate the experimental error of the measured fluorescence to one standard deviation.

3A). In 23 recordings of individual samples from five separate membrane preparations, channel gating started within 2 min of irradiation at 366 nm in the absence of applied tension. The channel opened during this "on" state with a conductance of 0.5 to 1 nS, which increased to 1.5 nS after application of a pressure gradient [Supporting Online Material (SOM) Text]. UV-induced openings of the channels consistently start after a lag period. This delay in activation is consistent with a comparable 2-min time scale observed in the absorption spectra for the neutral-to-zwitterionic isomerization to reach completion (Fig. 2B). Once activated, however, the channels continue to work. If the patch is then irradiated with visible light, the channel activity drops substantially within seconds and the channels switch off. The rapid deactivation, contrasted with the slow activation, suggests a critical polarity (dependent on the number of switches in the zwitterionic MC form) necessary for ion conduction through the hydrophobic pore of the homopentamer channel. It is not yet clear how many charges are necessary to open the pore.

Irradiation of the closed channel with a second UV cycle follows the same pattern as in the first cycle, and, after a lag period, channel openings are evident from current flow. Figure 3B shows representative channel activity during the last 40 s of each irradiation period. There are many channel opening events during the first UV period, whereas there are no channel openings at the end of the visible light treatment. The lower number of channel openings on the second UV treatment compared with the first correlates with the lower amount of the zwitterionic MC form seen in the absorption spectrum (Fig. 2C) after the first cycle of illumination.

In order to test the utility of the light-addressable nanovalve in controlling the exchange of solutes other than ions across a membrane, we conducted classical efflux experiments with a liposomal system containing a self-quenching fluorescent dye, calcein. The use of such calcein-loaded liposomes to monitor the permeability of liposomal membranes is a long-standing practice in the field of liposomal drug delivery (*29*). Because of the high local concentration inside the liposomes, the fluorescence intensity of calcein is low, but release from these liposomes results in dilution of the dye and consequently a large increase in fluorescence signal. Here, we adapted this method to study the gating of MscL under isoosmotic conditions. We reconstituted the modified photoactive MscL into liposomes and analyzed the response of the fluorescence signal to activation of the channel. In the case of the unidirectional nanovalve **1b**, some slow dye leakage (10%) was observed under ambient conditions without specific light stimulation, but 366-nm irradiation resulted in 43% release of liposomal content on the same time scale (Fig. 4A). As expected, proteoliposomes containing unmodified MscL did not release dye irrespective of illumination, nor did liposomes without protein.

Similar light-induced release of calcein under isoosmotic conditions was observed for the reversible nanovalve (Fig. 4B). In this case, however, the amount released was lower compared with the one-way switch even though the reconstitution conditions were the same. The major difference between the two photosensitive molecules is their hydrophobicity, which is a key parameter for inducing spontaneous gating of this otherwise mechanosensitive channel. The calculated hydrophobicity of the reversible switch is higher than that of the one-way switch. These results are nonetheless a clear step toward a practical, photogated nanoscale delivery system.

References and Notes

1. B. L. Feringa, *Molecular Switches* (Wiley, New York, 2001).
2. K. Ichimura, S.-K. Oh, M. Nakagawa, *Science* **288**, 1624 (2000).
3. C. Bertarelli, A. Bianco, F. D'Amore, M. C. Gallazzi, G. Zerbi, *Adv. Funct. Mater.* **14**, 357 (2004).
4. J. H. Folgering, J. M. Kuiper, A. H. de Vries, J. B. Engberts, B. Poolman, *Langmuir* **20**, 6985 (2004).
5. M. V. Alfimov, O. A. Fedorova, S. P. Gromov, *J. Photochem. Photobiol. A* **158**, 183 (2003).
6. I. Willner, S. Rubin, *Angew. Chem. Int. Ed.* **35**, 367 (1996).
7. S. Sukharev, A. Anishkin, *Trends Neurosci.* **27**, 345 (2004).
8. N. Levina *et al.*, *EMBO J.* **18**, 1730 (1999).
9. C. C. Cruickshank, R. F. Minchin, A. C. Le Dain, B. Martinac, *Biophys. J.* **73**, 1925 (1997).
10. B. Ajouz, C. Berrier, A. Garrigues, M. Besnard, A. Ghazi, *J. Biol. Chem.* **273**, 26670 (1998).
11. C. Berrier, A. Garrigues, G. Richarme, A. Ghazi, *J. Bacteriol.* **182**, 248 (2000).
12. P. Blount, S. I. Sukharev, M. J. Schroeder, S. K. Nagle, C. Kung, *Proc. Natl. Acad. Sci. U.S.A.* **93**, 11652 (1996).
13. C. C. Hase, A. C. Le Dain, B. Martinac, *J. Membr. Biol.* **157**, 17 (1997).
14. P. Blount, M. J. Schroeder, C. Kung, *J. Biol. Chem.* **272**, 32150 (1997).
15. X. Ou, P. Blount, R. J. Hoffman, C. Kung, *Proc. Natl. Acad. Sci. U.S.A.* **95**, 11471 (1998).
16. K. Yoshimura, A. Batiza, M. Schroeder, P. Blount, C. Kung, *Biophys. J.* **77**, 1960 (1999).
17. E. Perozo, A. Kloda, D. M. Cortes, B. Martinac, *J. Gen. Physiol.* **118**, 193 (2001).
18. G. Chang, R. H. Spencer, A. T. Lee, M. T. Barclay, D. C. Rees, *Science* **282**, 2220 (1998).
19. S. Sukharev, M. Betanzos, C. S. Chiang, H. R. Guy, *Nature* **409**, 720 (2001).
20. E. Perozo, D. M. Cortes, P. Sompornpisut, A. Kloda, B. Martinac, *Nature* **418**, 942 (2002).
21. J. Gullingsrud, K. Schulten, *Biophys. J.* **85**, 2087 (2003).
22. K. Yoshimura, A. Batiza, C. Kung, *Biophys. J.* **80**, 2198 (2001).
23. M. Sukhareva, D. H. Hackos, K. J. Swartz, *J. Gen. Physiol.* **122**, 541 (2003).
24. A. Anishkin, C. S. Chiang, S. Sukharev, *J. Gen. Physiol.* **125**, 155 (2005).
25. Materials and methods are available as supporting material on *Science* Online.
26. C. G. Bochet, *J. Chem. Soc. Perkin Trans. 1* 125 (2002).
27. R. Reinhard, B. F. Schmidt, *J. Org. Chem.* **63**, 2434 (1998).
28. The hydrophilicity of the 22nd position of MscL determines the magnitude of tension required for channel opening (*16*). When glycine is replaced by cysteine, the higher hydrophobicity of cysteine stabilizes the closed state, and actuation requires a tension high enough to rapture the patch. The nitrophenyl derivative in modified MscL **1b** increases hydrophobicity even further. Therefore, we expect the channel not to open in the dark even if tension is applied up to the breaking point of the patch. However, upon illumination, the generation of charge at the same position leads to spontaneous opening in the absence of applied tension.
29. R. R. C. New, *Liposomes: A Practical Approach* (IRL Press, Oxford, 1990), pp. 105–161.

30. We thank the MscL team in Groningen for their contribution, B. Poolman and D. Stavenga for critical reading of the manuscript, the neurobiophysics group in University of Groningen for making available the patch clamp facilities, and the Netherlands Organization for Scientific Research (NWO-CW) (B.L.F.) and the Materials Science Centre (MSC plus), University of Groningen (M.W. and B.L.F), for financial support.

Supporting Online Material

www.sciencemag.org/cgi/content/full/309/5735/755/DC1
Materials and Methods
SOM Text
Figs. S1 to S7
References and Notes

12 May 2005; accepted 14 June 2005
10.1126/science.1114760

Permanent El Niño–Like Conditions During the Pliocene Warm Period

Michael W. Wara, Ana Christina Ravelo,* Margaret L. Delaney

During the warm early Pliocene (~4.5 to 3.0 million years ago), the most recent interval with a climate warmer than today, the eastern Pacific thermocline was deep and the average west-to-east sea surface temperature difference across the equatorial Pacific was only 1.5 ± 0.9°C, much like it is during a modern El Niño event. Thus, the modern strong sea surface temperature gradient across the equatorial Pacific is not a stable and permanent feature. Sustained El Niño–like conditions, including relatively weak zonal atmospheric (Walker) circulation, could be a consequence of, and play an important role in determining, global warmth.

The low-latitude Pacific Ocean provides a substantial portion of the global atmosphere's sensible and latent heat and is thus a central driver of climate (*1*). Over the past 25 years, the mean equatorial Pacific sea surface temperature (SST) has increased by ~0.8°C (*2*), possibly in response to increasing greenhouse gas concentration (*3*). Changes in the tropical Pacific mean climatic state may influence the amplitude of interannual, or El Niño–Southern

Oscillation (ENSO), climate variability (*4*–*7*), which may in turn play a role in global warming (*8*). The tropical Pacific mean state can be influenced by extratropical conditions, where surface water is subducted and flows into the tropical thermocline (the steep subsurface vertical thermal gradient between warm surface and cooler deep waters) (*4*, *7*, *9*–*12*). Conversely, the mean state of the tropical Pacific could be determined by changes in ENSO variability itself (*13*, *14*). Overall, the mechanisms that control the mean state of the tropical Pacific are not fully understood, and predictions of future change in the mean state do not agree, probably because ENSO dynamics are not well-represented by most general circulation models (*15*). Thus, observational studies are needed to add additional constraints on the interplay between mean tropical conditions and global climate change.

Because instrumental (directly measured) records of climate change are relatively short (~100 years), geological records must be used to test theories that link long-term global climate change with tropical conditions (*7*). Characterizing conditions during times of global warmth requires investigation of older geologic periods that were substantially warmer than today. The most recent such interval is the Pliocene warm period [~4.5 to 3.0 million years ago (Ma)], which was characterized by ~3°C higher global surface temperatures relative to today (*16*). Although not a direct analogy of future transient global warming, the Pliocene warm period is a relevant natural experiment that can be used to understand processes contributing to long-term global warmth, because many boundary conditions were similar to today, including first-order ocean circulation patterns, the Earth's continental configuration, small Northern Hemisphere ice coverage, and atmospheric carbon dioxide concentrations (about 30% higher than pre-anthropogenic values) (*16*).

The equatorial west-to-east SST gradient and thermocline depth are intimately coupled parameters that exert a significant influence over both the mean state and variability of tropical Pacific climate (*4*, *7*). Today, tropical trade winds drive ocean currents westward, resulting in a thick, warm, mixed layer and deep thermocline in the western equatorial Pacific (WEP) and a thin, warm, mixed layer and shallow thermocline in eastern equatorial Pacific (EEP). The trades also drive surface water divergence and upwelling of warm water in the WEP and cold water in the EEP, where the thermocline is deep and shallow, respectively. Thus, the trade winds cause a west-to-east, or zonal, asymmetry in thermocline depth, SST (Fig. 1), and surface air pressure, which in turn strengthens the winds and further augments this asymmetry. The atmospheric circulation (Walker) cell, including easterly trade winds, rising air in the west, westerly winds aloft, and sinking air in the east, is a persistent feature of the tropical Pacific today; the magnitude of the zonal SST gradient is an excellent diagnostic of the strength of Walker circulation. Extreme temporary reductions in the zonal SST gradient and Walker circulation, or El Niño events, occur every 2 to 7 years and dramatically influence global climate by redistributing heat stored in the tropical Pacific to extratropical latitudes (*7*, *15*). Likewise, changes in the long-term SST gradient may have altered extratropical conditions for sustained periods of time in the past (*17*) and could also potentially influence global climate in the future (*9*).

To represent the east-west Pacific SST gradient, we used the difference in SST and $\delta^{18}O$ between Ocean Drilling Program (ODP) site 806 in the WEP and ODP site 847 in the EEP (Fig. 1). Our sites are ideally located to monitor changes in equatorial upwelling and thermocline depth (away from the confounding effects of the Peru-Chile upwelling system). At each site, we made paired $\delta^{18}O$ and magnesium-to-calcium ratio (Mg/Ca) measurements on foraminiferal shells (a surface-dwelling species) to construct a time series of $\delta^{18}O$ and SST [using the calibration of Dekens *et al.* (*18*)], with an average sampling interval of 10 kyear from 5.3 Ma to present (*19*). The SST and $\delta^{18}O$ records, smoothed to remove glacial-interglacial variability, indicate that from 5.3 to 1.7 Ma the west-to-east difference in both parameters (Fig. 2, A and B) was relatively small: the west was ~2°C colder and the east ~2°C warmer than modern SSTs, and the west-to-east SST difference was always less than 2°C, with an average of 1.5 ± 0.9 (Fig. 2C). After 2.5 Ma, SST in the EEP began gradually decreasing. At 1.7 Ma, WEP temperatures warmed by ~2°C over a 50-kyear period, while the EEP continued to cool. By 1.6 Ma, the modern zonal SST difference of ~4°C, equivalent to a Mg/Ca contrast of 35%, was established. Thereafter, the EEP gradually cooled, and the mean west-to-east SST difference for the last 1.6 myear was 5.1 ± 0.9°C.

Our results contradict a recent study (*20*), using the same methodology and site selection (*19*), that concluded that the EEP was cooler (not warmer) in the early Pliocene. In the Rickaby and Halloran study (*20*), SST changes over the last 5 myear were represented by six data points, with only one data point from the Pliocene warm period interval, and we suspect that aliasing of higher frequency (orbital scale) variability led those authors (*20*) to erroneously conclude that the EEP was colder in the Pliocene warm period than at present. Our higher temporal resolution study, using over 400 data points over the last 5 myear, provides a more accurate reconstruction of mean SST trends and firm evidence for warm SSTs in the EEP during the Pliocene warm period. Furthermore, warmer-than-present alkenone-based SST estimates from the EEP (*21*) and foraminiferal $\delta^{18}O$ records (Fig. 2A) (*16*, *22*, *23*) indicating reduced hydrographic differences across the Pacific during the Pliocene warm period are consistent with the interpretations of our Mg/Ca records (Fig. 2C).

To track changes in the mean thermocline depth at the EEP site, we used $\Delta\delta^{18}O$, the difference in $\delta^{18}O$ between surface-dwelling *Globoritalia sacculifer* (without sac) and *G. tumida* (355 to 425 μm) (Fig. 2A), which occupies the base of the photic zone (at ~100 m

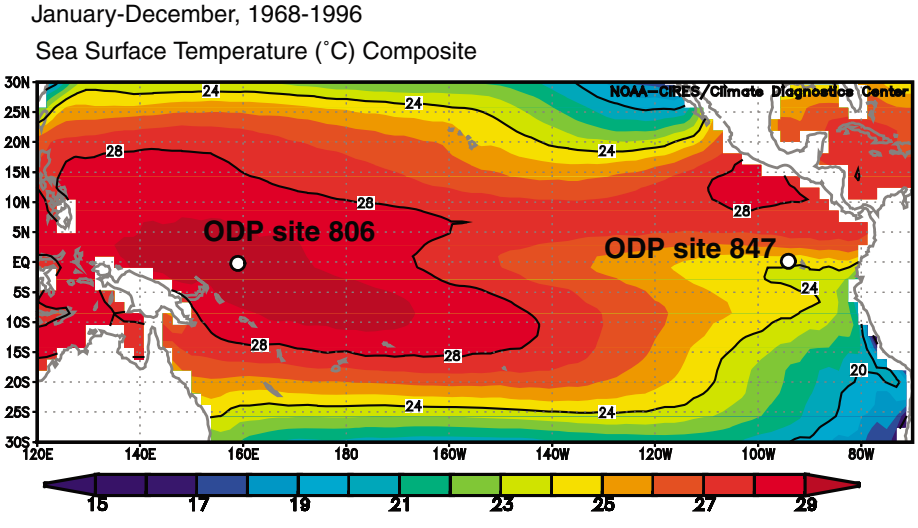


Fig. 1. Sites used in this study, ODP site 847 (0°N, 95°W, 3373-m water depth) and ODP site 806 (0°N, 159°E, 2520-m water depth), overlaid on a map of climatological mean SSTs in the tropical Pacific Ocean (*36*, *37*).

Ocean Sciences Department, University of California, Santa Cruz, CA 95064, USA.

*To whom correspondence should be addressed. E-mail: acr@es.ucsc.edu

depth) (*24*, *25*). Because there is a weak vertical salinity gradient in the EEP today, we assume that $\Delta\delta^{18}O$, on first order, represents the difference in temperature [ΔT between the surface and ~100m (Fig. 2D)]. High ΔT indicates that the cool thermocline water was shallow and above the base of the photic zone, whereas low ΔT indicates that cool thermocline water was deep and below the base of the photic zone. The increase in $\Delta\delta^{18}O$ of ~1.2 per mil (‰) (Fig. 2A) equates to an increase in ΔT of ~5°C (*26*) (Fig. 2D), indicating the presence of a warmer or deeper thermocline in the beginning of the Pliocene, significant shoaling or cooling of the thermocline from 5.3 to 3.5 Ma, and relatively constant conditions from 3.5 Ma to present.

Previous work, which supports our thermocline reconstructions, indicates that $\delta^{18}O$ differences between depth-stratified foraminiferal species (*22*, *27*), foraminiferal species assemblages (*27*), which are strongly correlated to thermocline depth (*25*), and Mg/Ca-derived subsurface temperatures (*20*) were similar on opposite sides of the basin during the warm Pliocene and became dissimilar by ~3.5 Ma. Taken together with our Mg/Ca-based evidence for a reduced west-to-east SST difference, these data indicate that the Pliocene warm period was not characterized by the typical west-to-east asymmetric conditions of the modern equatorial Pacific (Fig. 1). Rather, the Pliocene warm period had permanent El Niño–like conditions in several important aspects: Relative to today, the equatorial upwelling region of the EEP was warmer (Fig. 2B), the west-east SST difference along the equator was reduced (Fig. 2C), the thermocline in the EEP was deeper (Fig. 2D), and subsurface conditions were more symmetric across the tropical Pacific (*20*, *27*). These observations are consistent with each other: A warmer and/or deeper thermocline would have resulted in warmer SSTs in EEP upwelling regions and reduced zonal SST and surface air pressure gradients. The reduced pressure gradient would have caused the winds and Walker circulation to slacken, thereby reinforcing the effects of warmer thermocline waters (*28*). Weak Walker circulation would have influenced the position and intensity of extratropical high- and low-pressure centers (*29*) and therefore would have had far-reaching climatic effects. In fact, the global expression of Pliocene warmth resembles the teleconnection pattern of El Niño (*30*).

The observed El Niño–like mean state during the Pliocene warm period could be related to changes in the mean state of extratropical regions (*7*). Theoretically, reduced subtropical SST (*11*) or surface salinity (*12*) gradients could have resulted in a warmer and/or deeper tropical thermocline and, consequently, warm water upwelling in the EEP. Although there is observational evidence for warmer subtropical SSTs (*31*), more detailed information on subtropical SST and salinity gradients is needed. Alternatively, the mean state could have been influenced by processes within the tropics, such as changes in the character of short-term (ENSO) variability (*13*, *14*) perhaps due to the slightly different global boundary conditions, such as atmospheric CO_2 concentrations, of the early Pliocene compared to today.

Our sites alone cannot be used to determine whether the strong north-south SST gradients (Fig. 1) changed over time; future work detailing the spatial patterns of the tropical Pacific conditions could be used to test theories of what may have caused observed changes in equatorial conditions. In addition, future studies that try to differentiate between extratropical and within-tropical impacts on the mean state of the tropical Pacific should consider that several eastern boundary regions (California, Peru-Chile, West African margins), where cool upwelling occurs today, were significantly warmer before ~ 3 Ma (*21*, *32*), suggesting that the thermocline was deeper and/or warmer globally, and not just in the tropical Pacific.

The difference in timing between the decrease in thermocline depth before ~4.0 Ma (Fig. 2D) and the increase in zonal SST difference at ~1.7 Ma (Fig. 2C) could be explained by several factors. First, because the thermocline depth proxy, $\Delta\delta^{18}O$, depends on the $\delta^{18}O$ of *G. tumida*, which has a depth ecology at the base of the photic zone at ~100 m, it may not have been sensitive to additional shoaling above 100 m, which could have occurred after 4.0 Ma. Second, changes in salinity (and associated $\delta^{18}O$) of subsurface water could have a secondary effect on $\Delta\delta^{18}O$; thus, the effect of an increase in ΔT on $\Delta\delta^{18}O$ after 4.0 Ma could be masked by a synchronous decrease in salinity of subsurface water. Detailed records of paired Mg/Ca and $\delta^{18}O$ measurements on *G. tumida* are needed to better assess ΔT (*33*). And third, thermocline depth and SST are not linearly related (*4*), because the air-sea feedbacks that cause changes in Walker circulation become stronger as the ther-

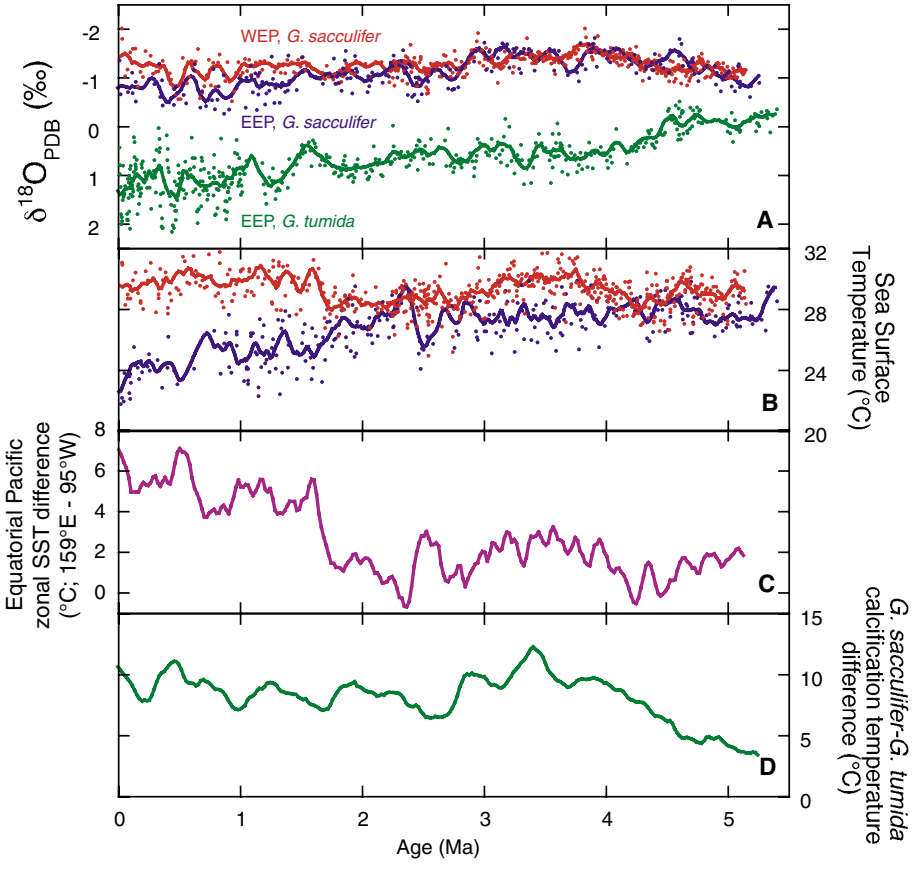


Fig. 2. Equatorial Pacific isotopic and temperature records. (**A**) oxygen isotope records of *G. sacculifer* (without sac, 355 to 425 μm) at ODP site 847 (blue) and 806 (red) as well as of *G. tumida* (355 to 425 μm) at ODP site 847 (green). (**B**) SSTs estimated from Mg/Ca measurements in *G. sacculifer* (without sac) from sites 847 (blue) and 806 (red). (**C**) The estimated zonal SST gradient on the equator between 159°E and 95°W. (**D**) The difference (Δ°C) between the calcification temperatures of *G. sacculifer* (without sac) and *G. tumida* at site 847 calculated by assuming that the difference between their $\delta^{18}O$ entirely reflects temperature and that $\Delta\delta^{18}O/0.21 = \Delta$°C. Heavy lines in all figures represent a 0.2-Ma Gaussian weighted running mean. Curves in (C) and (D) were calculated by using the smooth curves in (A) and (B).

mocline shoals. Thus, the increase in the zonal SST difference after ~1.7 Ma could indicate that tropical air-sea feedbacks (*34*) amplified the SST response to a changing thermocline once critical thermocline conditions were reached.

Mean tropical thermocline conditions can influence air-sea feedbacks that affect high-frequency climate variability (*4*, *15*); the amplitude of ENSO variability is dampened when the thermocline is deeper or warmer in the EEP (*5*, *10*). This effect applied to longer time scales may explain why permanent El Niño conditions during the Pliocene were accompanied by reduced-amplitude glacial-interglacial cycles; a deeper or warmer thermocline may have preconditioned the tropical system such that air-sea feedbacks needed to amplify small perturbations in solar forcing were weak. The establishment of Walker circulation at ~1.7 Ma coincides with the Pliocene-Pleistocene epoch boundary, after which the sensitivity of climate to solar forcing peaked (*16*).

Our study indicates that today's zonally asymmetric SST pattern and thermocline structure of the tropical Pacific are not stable over long time scales. Given the importance of tropical Pacific processes in modulating meriodional heat transport, these results indicate that in a warmer world, the ocean may accomplish redistribution of heat in a fundamentally different way. Thus, the Pliocene warm period provides a target and a test to climate models and theory and is an indication that climate feedbacks do not work to maintain the presently strong asymmetry across the Pacific under some circumstances. It may indicate that warming cannot continue indefinitely without substantial changes in the Walker circulation (*10*) and that changes in the subtropics, communicated through the thermocline, might cause a fundamental reorganization of the tropical Pacific ocean-atmosphere system (*4*, *10*). Depending on one's interpretation of the instrumental data from the tropical Pacific, a shift in the baseline tropical Pacific pattern may already be occurring (*2*, *4*, *5*, *8*).

References and Notes

1. M. A. Cane, *Science* **282**, 59 (1998).
2. M. J. McPhaden, D. Zhang, *Nature* **415**, 603 (2002).
3. U. Cubasch *et al.*, in *Climate Change 2001, The Scientific Basis*, J. T. Houghton, Y. Ding, Eds. (Cambridge Univ. Press, New York, 2001), pp. 525–582.
4. A. V. Fedorov, S. G. H. Philander, *J. Clim.* **14**, 3086 (2001).
5. A. V. Fedorov, S. G. Philander, *Science* **288**, 1997 (2000).
6. D. S. Battisti, A. C. Hirst, *J. Atmos. Sci.* **46**, 1687 (1989).
7. D.-E. Sun, T. Zhang, S.-I. Shin, *J. Clim.* **17**, 3786 (2004).
8. K. E. Trenberth, J. M. Caron, D. P. Stepaniak, S. Worley, *J. Geophys. Res.* **107**, 10.1029/2000JD000298 (2002).
9. D.-E. Sun, *J. Clim.* **16**, 185 (2003).
10. D.-E. Sun, J. Fasullo, T. Zhang, A. Roubicek, *J. Clim.* **16**, 2425 (2003).
11. G. Boccaletti, R. C. Pacanowski, S. G. H. Philander, A. V. Fedorov, *J. Phys. Oceanogr.* **34**, 888 (2004).
12. A. V. Fedorov, R. C. Pacanowski, S. G. H. Philander, G. Boccaletti, *J. Phys. Oceanogr.* **34**, 1949 (2004).
13. K. B. Rodgers, P. Friederichs, M. Latif, *J. Clim.* **17**, 3761 (2004).
14. A. Timmermann, *Global Planet. Change* **37**, 135 (2003).
15. M. A. Cane, *Earth Planet. Sci. Lett.* **230**, 227 (2005).
16. A. C. Ravelo, D. H. Andreasen, M. Lyle, A. O. Lyle, M. W. Wara, *Nature* **429**, 263 (2004), and references therein.
17. J. H. Yin, D. S. Battisti, *J. Clim.* **14**, 565 (2001).
18. P. S. Dekens, D. W. Lea, D. K. Pak, H. J. Spero, *Geochem. Geophys. Geosystems* **3**, 1022 (2002).
19. Materials and methods are available as supporting material on *Science* Online.
20. R. E. M. Rickaby, P. Halloran, *Science* **307**, 1948 (2005).
21. A. M. Haywood, P. Dekens, A. C. Ravelo, M. Williams, *Geochem. Geophys. Geosyst.* **6**, 10.1029/2004GC000799 (2005).
22. K. Cannariato, A. C. Ravelo, *Paleoceanography* **12**, 805 (1997).
23. A. C. Ravelo, M. W. Wara, *Oceanography* **17**, 22 (2004).
24. A. C. Ravelo, R. G. Fairbanks, *Paleoceanography* **7**, 815 (1992).
25. D. J. Andreasen, A. C. Ravelo, *Paleoceanography* **12**, 395 (1997).
26. We use a sensitivity of foraminiferal calcite ^{18}O to temperature of 0.21‰ $\delta^{18}O$ per °C from (*35*).
27. W. Chaisson, A. C. Ravelo, *Paleoceanography* **15**, 497 (2000).
28. S. G. H. Philander, A. V. Fedorov, *Paleoceanography* **18**, 1045 10.1029/2002PA000837 (2003).
29. F. B. Schwing, T. Murphee, P. M. Green, *Prog. Oceanogr.* **53**, 115 (2002).
30. P. Molnar, M. A. Cane, *Paleoceanography* **17**, 11-1 (2002).
31. H. Dowsett, J. Barron, R. Poore, *Mar. Micropaleontology* **27**, 13 (1996).
32. J. R. Marlow, C. B. Lange, G. Wefer, A. Roselle-Melé, *Science* **290**, 2288 (2000).
33. Although a detailed study of *G. tumida* needs to be performed, a low-resolution record of Mg/Ca measurements of *G. tumida* from the same site location (ODP site 847) shows cooling of the subsurface before 3.5 Ma (*20*), implying that our use of $\delta^{18}O$ of *G. tumida* as an indicator of temperature is appropriate and that changes in the $\delta^{18}O$ of seawater are probably negligible.
34. J. Bjerknes, *Mon. Weather Rev.* **97**, 163 (1969).
35. B. E. Bemis, H. J. Spero, J. Bjima, D. W. Lea, *Paleoceanography* **13**, 150 (1998).
36. E. Kalnay *et al.*, *Bull. Am. Meteorol. Soc.* **77**, 437 (1996).
37. Data are available at www.cdc.noaa.gov.

38. We thank M. Cane, G. Philander, A. Fedorov, and P. Molnar for helpful discussions; R. Franks for analytical support; and A. Schilla, C. Ziegler, and M. Flower for help with sample preparation and analysis. We thank the Ocean Drilling Program (ODP) for supplying samples. The ODP is sponsored by NSF and participating countries under the management of Joint Oceanographic Institutions (JOI), Incorporated. We thank NSF (grant to A.C.R.) for funding this work. Requests for data should be sent to A.C.R.

Supporting Online Material

www.sciencemag.org/cgi/content/full/1112596/DC1
SOM Text
Materials and Methods
Tables S1 to S3
References and Notes

22 March 2005; accepted 14 June 2005
Published online 23 June 2005;
10.1126/science.1112596
Include this information when citing this paper.

Embryos of an Early Jurassic Prosauropod Dinosaur and Their Evolutionary Significance

Robert R. Reisz,[1]* Diane Scott,[1] Hans-Dieter Sues,[2] David C. Evans,[1] Michael A. Raath[3]

Articulated embryos from the Lower Jurassic Elliot Formation of South Africa are referable to the prosauropod *Massospondylus carinatus* and, together with other material, provide substantial insights into the ontogenetic development in this early dinosaur. The large forelimbs and head and the horizontally held neck indicate that the hatchlings were obligate quadrupeds. In contrast, adult *Massospondylus* were at least facultatively bipedal. This suggests that the quadrupedal gait of giant sauropods may have evolved by retardation of postnatal negative allometry of the forelimbs. Embryonic body proportions and an absence of well-developed teeth suggest that hatchlings of this dinosaur may have required parental care.

Prosauropod dinosaurs appeared during the early Late Triassic (*1*, *2*) and became the dominant large herbivores in Late Triassic and Early Jurassic continental ecosystems (*3*) [220 to 183 million years ago (Ma)]. The prosauropod *Massospondylus carinatus* Owen, 1854 is known from numerous well-preserved specimens from many localities in the Lower Jurassic Elliot and Clarens formations of South Africa and Lesotho (*4*, *5*). It is represented by many articulated skeletons that form an extensive growth series. Here we describe articulated embryonic skeletons referable to *Massospondylus* and provide evidence that the quadrupedal gait in sauropods may have evolved through paedomorphosis.

A cluster of six subspherical eggs (6 cm in maximum diameter) was collected from the *Massospondylus* Range Zone of the upper Elliot Formation in Golden Gate Highlands National Park in South Africa (*6*, *7*). During preparation, we identified embryonic skeletal material in the bottom halves of five of the eggs; the sixth egg seems to have hatched.

[1]Department of Biology, University of Toronto at Mississauga, Mississauga, ON L5L 1C6, Canada. [2]Department of Paleobiology, National Museum of Natural History, Smithsonian Institution, Washington, DC 20013–7012, USA. [3]Bernard Price Institute for Palaeontological Research, University of the Witwatersrand, Wits 2050, Johannesburg, South Africa.

*To whom correspondence should be addressed. E-mail: rreisz@utm.utoronto.ca

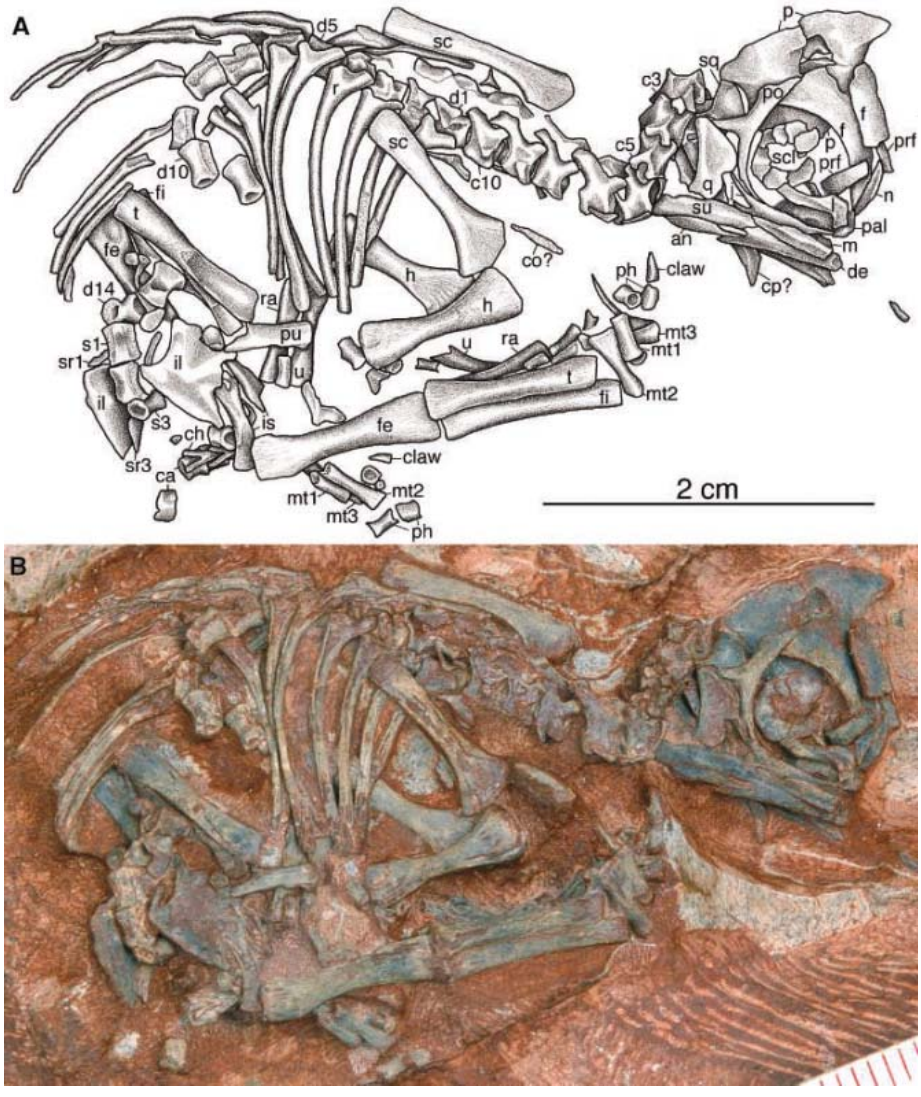


Fig. 1. (**A** and **B**) An articulated embryonic skeleton of *Massospondylus* (BP/1/5347A). an, angular; c3 to c10, cervical vertebrae; ca, caudal vertebra; ch, hemal arch; co, coracoid; cp?, cultriform process?; de, dentary; d1 to d14, dorsal vertebrae; f, frontal; fe, femur; fi, fibula; h, humerus; il, ilium; is, ischium; l, lacrimal; m, maxilla; mc, metacarpals; mt1 to mt3, metatarsals; n, nasal; p, parietal; pal, palatine; ph, phalanges; pm, premaxilla; po, postorbital; prf, prefrontal; pt, pterygoid; pu, pubis; q, quadrate; r, rib; ra, radius; s1 to s3, sacral vertebrae; sc, scapula; scl, scleral ring; sr1 to sr3, sacral ribs; sq, squamosal; su, surangular; t, tibia; u, ulna.

Other known fossil dinosaur eggs containing articulated embryonic skeletons are Late Cretaceous in age. Although the precise developmental stage of the embryos could not be determined, the articulated skeleton completely fills the egg and together with the advanced level of ossification (as indicated by the presence of the ossified stapes and fourth trochanter on the femur) suggests that the animals were close to hatching (*8*, *9*). Numerous skeletal features indicate that these embryos represent a prosauropod dinosaur: The first dentary tooth (alveolus) is set back from the anterior end of the bone; a lateral ridge is present on the dentary; the infratemporal fenestra extends below the orbit; the neck is horizontal rather than S-curved; the posterior dorsal centra are longer than they are tall; and humeral length exceeds 55% of that of the femur (*3*). We refer the embryos to *Massospondylus* based on a cranial autapomorphy, the maximum transverse width of the skull exceeding its height by at least 10% (*10*). *Massospondylus* is the most common sauropodomorph in this stratigraphic interval; two uncollected adult skeletons of this taxon have been found at the egg-producing locality, and several other skeletons are exposed within 500 m (*11*).

The embryonic contents of two of the six eggs have been completely prepared. One skeleton is preserved curled up and in articulation (Fig. 1). The skull has been slightly telescoped in the snout region before fossilization, with the anterior part of the mandible, the premaxilla, and the anterior end of the maxilla having been lost. The skull is virtually complete in the second embryo, and the position of various postcranial elements suggests that this skeleton was also articulated and curled up (Fig. 2). This skull is exposed in dorsal view, and the lower jaws have been shifted slightly forward, exposing their alveolar regions and symphysis. One fragment that may represent a partially erupted tooth is preserved in the dentary; all other alveoli are empty.

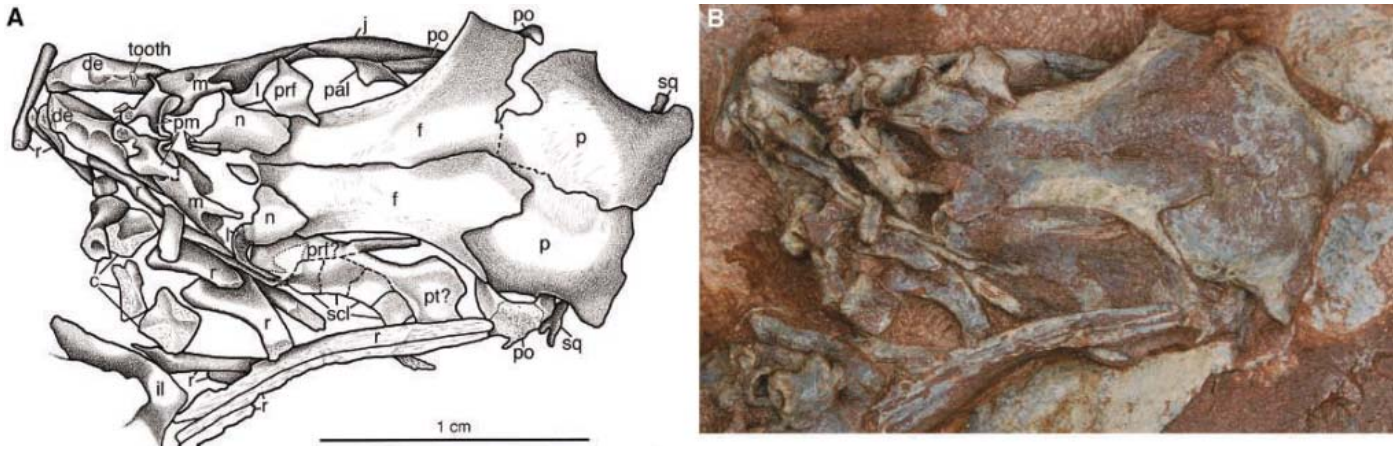


Fig. 2. (**A** and **B**) Embryonic skull and postcranial elements of *Massospondylus* (BP/1/5347A). The embryonic membrane is preserved inside the egg immediately to the right of the skull. Abbreviations are the same as in Fig. 1.

Embryonic skull structure (Fig. 3B) is consistent with referral to *Massospondylus*. The posterior part of the maxilla forms a distinct, slender process that underlies the jugal. The tall lacrimal is visible in dorsal view. A long posterodorsal process of the prefrontal is identical to that seen in other skulls of *Massospondylus*. The antorbital fenestra is tall and almost triangular in outline. The external naris is large in the embryo but not as tall as the antorbital fenestra. The nasal is small, the frontal is long, and the large, dome-like parietals are feebly emarginated for the upper temporal fenestra. In contrast, the largest known skull of *Massospondylus* (specimen BP/1/4934) has an elongate nasal, a relatively short frontal, and the parietals are strongly emarginated and form a median crest. Other smaller specimens show intermediate stages between the two extremes (*10*). The embryonic skulls preserve a nearly complete ring of scleral ossicles in the proportionately enormous orbit (the diameter is 39% of the reconstructed skull length). In addition, a stapes is still in its original position in one of the embryonic skulls; its distal end is slightly expanded, as in adults.

The embryonic postcranial skeleton generally conforms to that in *Massospondylus* but differs markedly in its proportions. The atlas-axis complex could not be exposed. The remaining eight cervical vertebrae are short with delicate ribs, in contrast to the elongate cervicals and cervical ribs in adults. Juvenile skeletons show intermediate stages in cervical development. Thirteen dorsals are preserved with most ribs still in their original positions. Damage in the posterior region of the series has disrupted the arrangement of ribs, but a tiny posterior left dorsal rib is preserved just anterior to the pelvis. The rod-like first sacral rib extends to the anterior edge of the iliac blade; its proportions support the hypothesis that the first sacral vertebra was a modified dorsal (*5*). The anterior caudals are substantially shorter than the dorsals, and their hemal spines form short, V-shaped chevrons.

Both pectoral and pelvic girdles are exposed. The scapula is tall and slender. Most of the right coracoid was lost during fossilization. There appear to be no ossified sternals. The iliac blade is well preserved; its preacetabular process is poorly developed and the supra-acetabular crest is absent (i.e., not ossified). The ischium and pubis are slender and short, about one-half the length of the femur.

The limb bones are well preserved, but the texture of their external surfaces indicates the presence of substantial cartilaginous "epiphyseal" caps. All forelimb elements are relatively longer than those in the adults, including the metacarpals and proximal phalanges, but this is consistent with their ontogenetic trajectories. The right femur has a slightly inturned proximal head and a greatly expanded distal end. The left femur shows the presence of an ossified fourth trochanter. The posterior surface of the distal end of the left femur has an intercondylar fossa. The proximal head of the tibia is not as wide as the distal end of the femur, whereas the fibula is relatively broad, consistent with the generally columnar appearance of the hindlimb. In the manus, metacarpals I to III and at least two phalanges are ossified, as are metatarsals I to III and some phalanges of the pes, including a complete second digit.

Massospondylus reached a total body length of up to 5 m (*5*). The large number of complete skeletons permits analysis of relative growth in the skull, vertebrae, and limbs (*12*). We made six bivariate comparisons using femur length as the standard variable (Fig. 4), which correlates well with overall body size in terrestrial vertebrates (*13*). Regression analyses show that the tibia and dorsal vertebrae in *Massospondylus* grew isometrically with reference to the femur. Both the humerus and the ulna show negatively allometric growth during ontogeny. The strongest negative allometry is observed in the skull, and the strongest positive allometry is seen in the length of the cervical vertebrae.

Relative incremental growth also accounts for proportional differences between the embryonic skulls and those of the adult. In embryonic skulls, the nasal is shorter than either the frontal or parietal, and the maxilla and premaxilla are also quite small relative to the rest of the skull. The known series of skulls of *Massospondylus* shows gradual elongation of the nasal, an increase in the relative length and height of the maxilla and premaxilla, and an increase in the size of the narial and antorbital

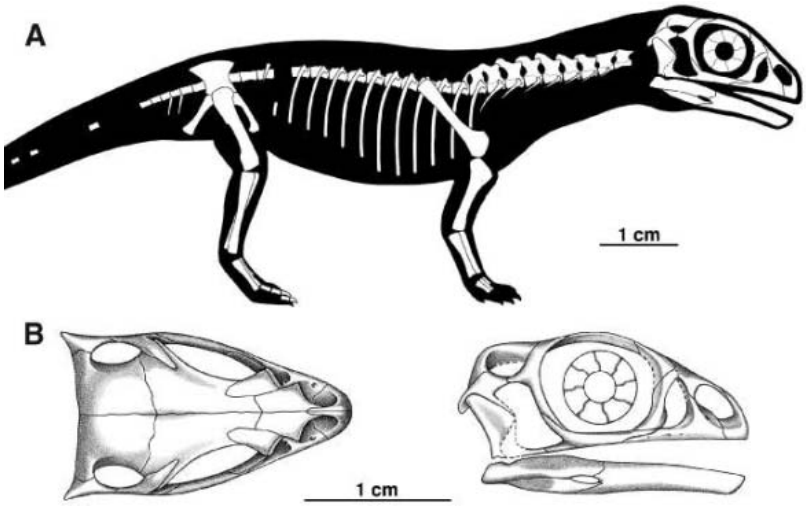


Fig. 3. Reconstructions of *Massospondylus* embryos. (**A**) An articulated skeleton in lateral view; the horizontally held neck is shown at maximum dorsiflexion. The total length of the tail could not be determined; the estimated minimum length is shown. Estimated snout-vent length of the embryo is 8.1 cm. (**B**) An embryonic skull in dorsal and lateral views.

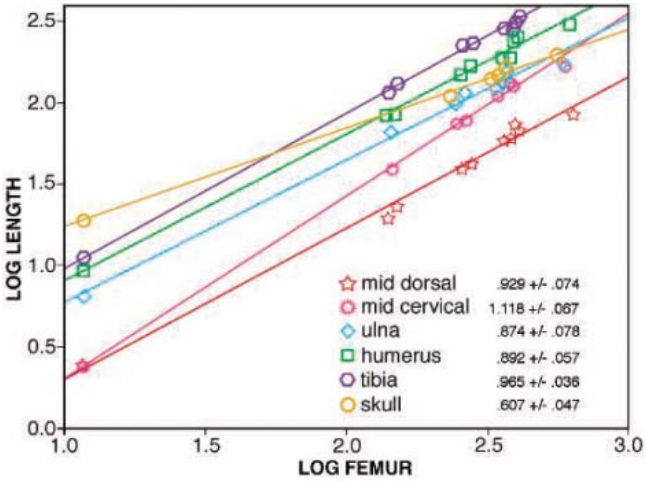


Fig. 4. Relative growth in the skeleton of *Massospondylus*. Regression analysis shows growth trajectories of various parts of the skeleton relative to the length of the femur. Eight skeletons, including the embryo, were sufficiently complete for this analysis, their femora showing a 47-fold increase in length in the series. Several otherwise superbly preserved specimens could not be used because they lacked a femur. One of the specimens (BP/1/4934) used in this analysis had an incomplete femur, and we estimated its total length by using the fourth trochanter as a landmark for comparisons with complete isolated femora at a similar level of development. Allometric coefficient values that are significantly less than 1.0 indicate negative allometry, as seen in the skull and forelimb, whereas values that are significantly greater than 1.0 denote positive allometry, as seen in the neck.

openings as the antorbital region of the skull becomes increasingly longer (*10*). Associated with this change is the gradual emargination of the frontals and parietals along the edges of the upper temporal fenestrae for the increase in the attachment areas for the adductor jaw musculature. The quadrate also shows a gradual relative increase in height, possibly related to the increase in the adductor jaw muscles. Finally, as in other vertebrates, the relative size of the orbit decreases throughout ontogeny. Despite these changes, the embryo maintains the diagnostic skull width-to-height ratio of *Massospondylus*.

The proportionately enormous skull; the long, horizontally held neck; the proportionately long forelimbs; and the small caudals with weakly developed transverse processes and hemal spines all indicate that the hatchlings of *Massospondylus* were obligate quadrupeds (Fig. 3). This is in contrast to the body proportions of adult *Massospondylus*, which are characterized by a small head, short forelimbs, and robust caudals with large transverse processes and hemal arches. As in most prosauropods, these adult proportions indicate at least facultatively bipedal locomotion (*14*, *15*). The changes from a sauropodlike condition with long forelimbs (*16*) and quadrupedal posture in early ontogenetic stages of *Massospondylus* to the plesiomorphic condition of short forelimbs and bipedal posture in the adult suggest that the quadrupedal posture of sauropods (*17*) may have evolved through paedomorphosis, the retention of early ontogenetic features in the adult, as first suggested by Bonaparte and Vince (*18*).

Modification of the sauropodomorph neck may have been correlated with the evolution of quadrupedality in this clade. The reconstructed quadrupedal posture for the embryo is at least in part related to the structure of the neck. As in most sauropodomorphs (*19*), the neck of *Massospondylus* was held more or less horizontally. This condition represents an autapomorphy for Sauropodomorpha and differs from the plesiomorphic condition of an S-curved neck in other dinosaurs and their immediate outgroups among Ornithodira (*20*). The S-curved neck is associated with their bipedal posture and the need for bringing the head closer to the center of gravity of the body. The presence of a horizontally held neck in the prehatching stages of basal sauropodomorphs like *Massospondylus* may have constrained them to a quadrupedal posture, one that required long forelimbs.

The combination of the body proportions and poorly developed dentition suggest that the hatchlings may have required parental care (*21*). The diminutive ventral elements of the pelvic girdle, small caudal vertebrae, and relatively enormous head of the *Massospondylus* embryos suggest it would have been difficult for the hatchlings to move around efficiently. The virtual absence of teeth in these embryos is another indicator of altricial behavior. Only a single possible tooth fragment is preserved in the two skulls, whereas other delicate, loosely attached elements were preserved largely undisturbed. Even if most of the teeth were poorly mineralized or lost postmortem, they were not well suited for feeding. If this interpretation is correct, these embryos provide early evidence of altricial behavior in a nonavian dinosaur.

References and Notes

1. M. C. Langer, F. Abdala, M. Richter, M. J. Benton, *Comp. Rend. Acad. Sci. Paris Sér. 2* **329**, 511 (1999).
2. A. M. Yates, *J. Syst. Palaeontol.* **1**, 1 (2003).
3. P. M. Galton, P. Upchurch, in *The Dinosauria*, D. B. Weishampel, P. Dodson, H. Osmólska, Eds. (Univ. of California Press, Berkeley, CA, 2004), ed. 2, pp. 232–258.
4. J. W. Kitching, M. A. Raath, *Palaeontol. Afr.* **25**, 111 (1984).
5. M. R. Cooper, *Occ. Papers Natl. Mus. Monum. (Rhodesia) B* **6**, 689 (1981).
6. The clutch of eggs was collected by J. W. Kitching in 1978 along a road-cut at the locality called Rooidraai (Red Corner), along the main road connecting the towns of Clarens and Phuthaditjhaba (28°30.565′S, 28°37.376′E).
7. J. W. Kitching, *Palaeontol. Afr.* **22**, 41 (1979).
8. M. W. J. Ferguson, in *Biology of the Reptilia*, C. Gans, F. Billett, P. F. A. Maderson, Eds. (Wiley, New York, 1985), vol. 14, pp. 329–491.
9. J. M. Starck, in *Avian Growth and Development*, J. M. Starck, R. E. Ricklefs, Eds. (Oxford Univ. Press, Oxford, 1998), pp. 59–88.
10. H.-D. Sues, R. R. Reisz, S. Hinic, M. A. Raath, *Ann. Carnegie Mus.* **73**, 239 (2004).
11. These partially exposed skeletons of *Massospondylus* are used by the Golden Gate Highlands Park rangers for their guided tours and cannot be collected.
12. Materials and methods are available as supporting material on *Science* Online.
13. P. J. Currie, *Can. J. Earth Sci.* **40**, 651 (2003).
14. M. F. Bonnan, *J. Vertebr. Paleontol.* **23**, 595 (2003).
15. A. M. Yates, J. W. Kitching, *Proc. R. Soc. London Ser. B.* **270**, 1753 (2003).
16. M. J. Carrano, *Paleobiology* **26**, 489 (2000).
17. J. A. Wilson, P. C. Sereno, *Mem. Soc. Vertebr. Paleontol.* **5**, 1 (1998).
18. J. F. Bonaparte, M. Vince, *Ameghiniana* **16**, 173 (1979).
19. K. A. Stevens, J. M. Parrish, *Science* **284**, 798 (1999).
20. P. C. Sereno, *Mem. Soc. Vertebr. Paleontol.* **2**, 1 (1991).
21. J. M. Starck, R. E. Ricklefs, in *Avian Growth and Development*, J. M. Starck, R. E. Ricklefs, Eds. (Oxford Univ. Press, Oxford, 1998), pp. 3–26.

22. We thank C. Vasconcelos, A. Yates, and C. Kelly for information and discussions; B. Rubidge and R. Smith for access to specimens and assistance; and the staff of the Golden Gate Highlands National Park. This work is dedicated to the memory of Dr. James Kitching. Supported by the University of Toronto, the Natural Sciences and Engineering Research Council of Canada, the National Geographic Society, and the Paleo-Anthropology Scientific Trust (South Africa).

Supporting Online Material

www.sciencemag.org/cgi/content/full/309/5735/761/DC1
Materials and Methods
Tables S1 and S2
References and Notes

17 May 2005; accepted 23 June 2005
10.1126/science.1114942

Pesticide Resistance via Transposition-Mediated Adaptive Gene Truncation in *Drosophila*

Yael T. Aminetzach, J. Michael Macpherson, Dmitri A. Petrov*

To study adaptation, it is essential to identify multiple adaptive mutations and to characterize their molecular, phenotypic, selective, and ecological consequences. Here we describe a genomic screen for adaptive insertions of transposable elements in *Drosophila*. Using a pilot application of this screen, we have identified an adaptive transposable element insertion, which truncates a gene and apparently generates a functional protein in the process. The insertion of this transposable element confers increased resistance to an organophosphate pesticide and has spread in *D. melanogaster* recently.

The *Drosophila* genome contains a large number of active transposable element (TE) families that generate new TE insertions (*1*, *2*). Many such TEs are deleterious at least partly because ectopic recombination among them scrambles chromosomes (*3*, *4*). Thus, many new TE insertions cannot reach high population frequencies unless they either recombine infrequently (*4*) or they lead to a sufficiently beneficial change to overcome the disadvantage of ectopic recombination.

Department of Biological Sciences, Stanford University, 371 Serra Mall, Stanford, CA 94305, USA.

*To whom correspondence should be addressed. E-mail: dpetrov@stanford.edu

To search for unusually frequent and therefore putatively adaptive TE insertions, we conducted a population survey of all of the 16 identified insertions of long interspersed element (LINE)–like *Doc* TEs (*5*) located in regions of high recombination in the sequenced *D. melanogaster* genome (*4*). All *Doc* elements except one (*Doc1420*) appeared to be subject to strong purifying selection. *Doc1420* occurred unusually frequently (*4*) despite being neither unusually short nor unusually divergent, which suggests that it either generates or is closely linked to an adaptive mutation.

Doc1420 is very frequent worldwide (~80%) and is much more rare (G test; $P = 0.0001$) in putatively ancestral African pop-

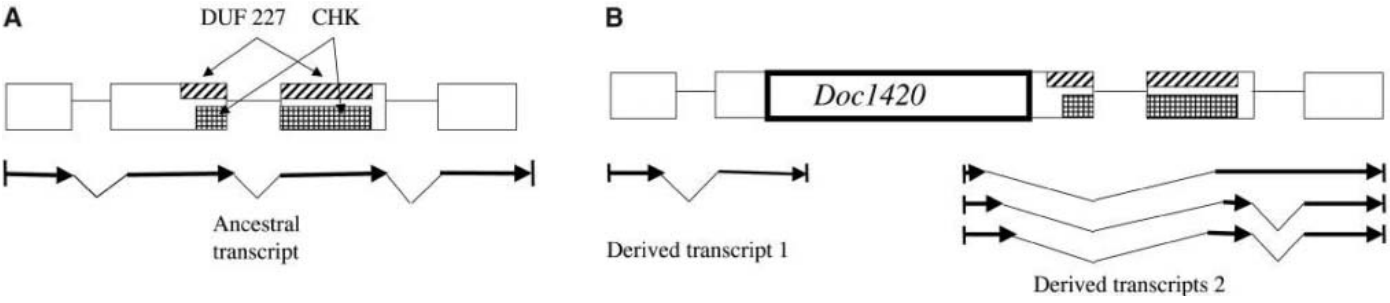


Fig. 1. Structure of *CHKov1*. (**A**) The structure of the ancestral transcript (before the insertion of *Doc1420*). The four exons are represented by rectangles. The transcript is depicted with black arrows. The striped and crosshatched areas represent PFAM domain DUF227 and SMART domain CLK, respectively. (**B**) Display of the altered transcripts in the strain possessing *Doc1420*. The first altered transcript contains the first exon, part of the second exon on the 5′ side of *Doc1420*, and ends 391 to 394 bp inside the *Doc1420* sequence. Identified transcripts in the second set start 3465 bp inside *Doc1420*; contain a short region of variable length (21, 38, and 77 bp in the three sequenced cases); they splice out, respectively, 1555, 1685, and 1549 bp regions containing part of the second exon on the 3′ side of *Doc1420* and part of the third exon; and they proceed until the end of the gene. In addition, one of the sequenced transcripts fails to splice out the intron between the third and fourth exons.

ulations of *Drosophila* in Zimbabwe and Malawi (table S1). This pattern is consistent with a genetic linkage between *Doc1420* and an adaptation associated with global expansion of *D. melanogaster* out of Africa.

To investigate the possible adaptive effects of *Doc1420*, we examined its effects on neighboring genes. *Doc1420* interrupts a predicted gene, *CG10618* (which we named *CHKov1*). *CHKov1* contains four exons [of length 175, 494, 367, and 197 base pairs (bp)], and *Doc1420* has inserted into the second exon (*6*). *CHKov1* appears to be functional; the *D. yakuba* ortholog of *CHKov1* [*D. yakuba* diverged from *D. melanogaster* ~5 million years ago (*7*)] shows conservation of all six intron junctions and of the predicted open reading frame. Moreover, there is a significant lack of amino acid substitutions (K_a), compared with the number of synonymous substitutions (K_s) between these two sequences (K_a/K_s = 0.1332; 95% confidence interval is 0.0486 to 0.3648), indicating that purifying selection has been preserving the sequence of the *CHKov1* protein for the past ~10 million years.

The *Doc1420* insertion in *CHKov1* generates two sets of altered transcripts (Fig. 1B) (*6*). In the presence of *Doc1420*, the transcript containing all four exons (and thus the original protein) appears to be absent. *CHKov1* and its paralogs share the PFAM domain DUF227 (amino acids 94 to 321), whose function is unknown (*8*), and a SMART domain CHK (amino acids 130 to 321), with a putative choline kinase function (*9*). The developmental profiles (*10*) of several of the closest paralogs of *CHKov1* have a consistent pattern of expression: high levels of expression in larvae and adults and low levels of expression in the embryo and pupae (fig. S2). This pattern is consistent with involvement of the paralogs of *CHKov1* (and by implication, with *CHKov1* itself) in digestion or detoxification. The altered transcripts (Fig. 1B) lack most of the conserved PFAM and SMART domains, which suggests that the original enzymatic function of *CHKov1* is likely to be lost in the presence of *Doc1420*.

If *Doc1420* is adaptive (or linked to an adaptive mutation), we expect to find the signature of an incomplete selective sweep: a sharp reduction of variability among the alleles linked to *Doc1420*. We tested this prediction by sequencing two regions [1.9 kilobases (kb) in the 5′ direction and 1.5 kb in the 3′ direction] immediately adjacent to *Doc1420* in an unstratified sample of 43 isofemale North American strains and seven isofemale Zimbabwe strains. We also sequenced this region in a single isofemale strain of *D. simulans*.

The haplotype structure near *Doc1420* (Fig. 2) strongly supports the hypothesis of a recent incomplete selective sweep. We found only six distinct haplotypes among the 34 alleles containing the *Doc1420* insertion, whereas each one of the 23 alleles without *Doc1420* is a unique haplotype. We conducted a series of coalescent simulations (*6*) to demonstrate that this structure is unexpected under neutrality. In 10^5 simulations, we failed to generate any samples with fewer than 18 distinct haplotypes (compared with the observed six distinct haplotypes) linked to a polymorphism as frequent as *Doc1420* [$P < 0.00001$, assuming recombination rate of 3.3 cM/Mbp (*11*); $P = 0.0009$, assuming no recombination].

If an incomplete selective sweep is associated with *Doc1420,* its signature should decay at increasing distances from *Doc1420*. Indeed, at a distance of ~18 kb in both the 5′ and 3′ directions from *Doc1420* (fig. S1), neutrality cannot be rejected, whereas at the two closer regions (15 kb in the 5′ direction and 11 kb in the 3′ direction from *Doc1420*), we found a modest signature of positive selection ($P < 0.05$ in each case). These results suggest that the incomplete selective sweep is centered at or very near to *Doc1420*.

We can use the rate of decay of the haplotype structure to estimate (*12*) that the latest incomplete selective sweep in this region occurred ~500 to 2400 generations ago. If one assumes 10 to 20 generations per year, this translates into ~25 to 240 years ago. The spread of *Doc1420* in the worldwide population of *D. melanogaster* appears at about the same time that drastic anthropogenic changes in the environment occurred, and possibly concurrently with the worldwide expansion of *D. melanogaster* out of Africa.

What is the adaptive effect that might be responsible for this recent expansion of the *Doc1420*-containing allele? We deduced that changes in *CHKov1* (a putative choline kinase) might affect choline metabolism in general and possibly the function of acetylcholine esterase. Given that acetylcholine esterase is the target of several types of pesticides, including organophosphates, we hypothesized that *Doc1420* insertion into *CHKov1* might confer resistance to organophosphates.

To test this possibility, we used repeated backcrosses to generate two *D. melanogaster* strains, with largely identical genetic backgrounds except for that at *CHKov1*, where one strain [*Doc+* (intro)] carried the insertion of *Doc1420* and the other [*Doc–* (intro)] lacked it (*6*). We assayed these two strains and the two parental strains for resistance to a commonly used organophosphate pesticide [azinphos-methylphosphate (AZM)]. The results (Fig. 3) indicate that the presence of *Doc1420* is associated with increased resistance to organophosphates in both the parental and the introgression strains. *Doc+* (introgression) has substantially lower mortality in this assay (20%) than does *Doc–* (intro) (68%) ($P < 0.001$).

To understand the history of this locus more completely, we investigated the molecular evolution of *CHKov1* and *CHKov2* (a paralog of *CHKov1* located immediately on the 5′ side, also known as *CG10675*). We specifically asked whether the *Doc1420*-containing alleles have evolved in a pattern consistent with a complete knockout of *CHKov1* or whether there is evidence of functionality of any of the resulting transcripts. We used the McDonald-Kreitman test (*13*), which tests the equality of

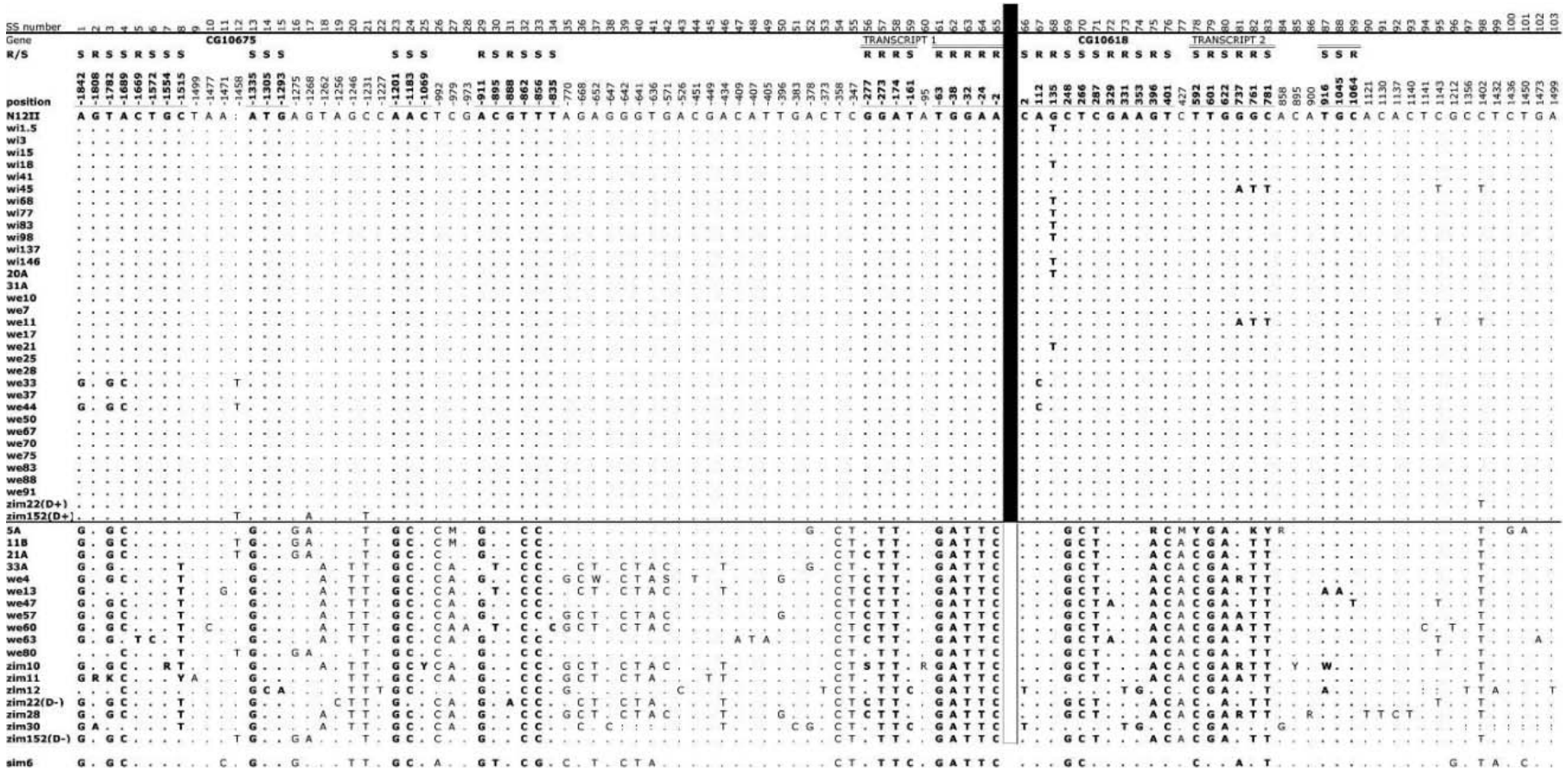


Fig. 2. Sequence of the 3.4-kb region flanking *Doc1420*. The figure shows the segregating sites (SS) within the 3.4-kb region flanking the *Doc1420* insertion. The SS number and the distance from *Doc1420* are at the top. The SS within coding regions are in bold and are identified as replacement (R) or synonymous (S) polymorphisms. The SS within *CHKov1* map to either derived transcript 1 (TRANSCRIPT 1) or to derived transcript 2 (TRANSCRIPT 2). *Doc1420* is shown as a black rectangle; the absence of Doc1420 is shown as an empty rectangle. The state for all of the segregating sites in the *D. simulans* sequence is shown at the bottom.

the ratio of amino acid and synonymous polymorphisms to the ratio of the number of amino acid and synonymous substitutions expected under a neutral model of evolution. This test (Table 1) revealed no violations of neutral expectations either for *CHKov2* or for the haplotypes of *CHKov1* lacking *Doc1420*. In contrast, haplotypes of *CHKov1* bearing *Doc1420* exhibited excess amino acid replacement polymorphism (Table 1), but only within the first and not the second set of derived transcripts (Fig. 1B). This excess is largely due to seven apparently derived amino acid polymorphisms (segregating sites 57, 58, 61, 62, 63, 64, and 65 in Fig. 2). Moreover, at these sites the derived states are fixed within the alleles containing *Doc1420*, whereas the ancestral states are fixed within the alleles lacking *Doc1420*. Additional sequencing of the transcript-1 region of *CHKov1* in 32 strains from Asia, Europe, Australia, and South America (table S3) confirmed this pattern. Thus, it appears that these amino acid changes have been newly generated within an allele containing *Doc1420*.

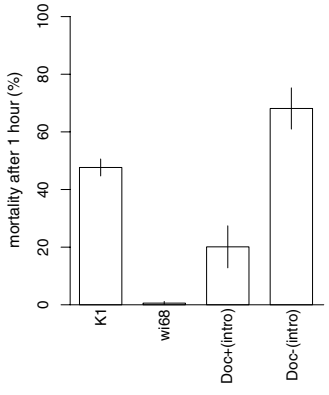


Fig. 3. Pesticide sensitivity assays. The average mortality in the presence of AZM for the four studied strains averaged over three independent experiments. The time of exposure and the dosage of AZM were chosen to achieve ~50% mortality of the more susceptible parental strain (*K1*). The error bars represent standard errors.

These seven new amino acid changes cluster together at the C terminus of the putative protein derived from derived transcript 1 (Fig. 1B and Fig. 2). Overall, they exchange primarily hydrophobic with primarily hydrophilic amino acids. In six out of seven cases, the changes are to either arginine or asparagines. No pattern of adaptive evolution is evident in the sequences on the 3′ side of *Doc1420*. These observations strongly suggest that positive natural selection has acted at the coding level of the new truncated polypeptide generated by the truncation of *CHKov1* by *Doc1420* (Fig. 1B; derived transcript 1). By implication, this new protein is likely to be functional.

Although the spread of the *Doc1420*-containing allele apparently took place 25 to 240 years ago, this allele contains eight independent changes, which suggests that it is much older and had undergone substantial evolution before its recent expansion. Indeed, the divergence of *Doc1420* from the consensus *Doc* sequence at 11 positions implies that *Doc1420* inserted ~90,000 years ago and certainly more than 240 years ago [G test, $P = 0.0004$; assuming 3×10^{-8} substitutions per bp per year (*14*)]. Further substantiating this claim, the pairwise divergence per nucleotide between the *Doc1420*-containing and *Doc1420*-lacking alleles (0.012 bp^{-1}) is more than twice as large ($P < 0.05$, as determined by bootstrapping) as the divergence among the alleles lacking *Doc1420* (0.005 bp^{-1}). The *Doc1420*-containing allele appears unusually old, even if the analysis is limited only to synonymous sites in *CHKov1* and *CHKov2* (table S2).

We propose that at some point in the past, one allele of *CHKov1* went through a number of drastic changes: first the insertion of *Doc1420* generated a functional truncated protein, which subsequently underwent rapid amino acid evolution. The final allele expanded 25 to 240 years ago in the worldwide population of *D. melanogaster*, with the aid of positive natural selection apparently by resistance to organophosphates. We speculate that the *Doc1420*-containing allele has been evolving in an isolated population of *D. melanogaster* for a substantial length of time, possibly ~90,000 years. Thus, although the recent expansion of the *Doc1420*-containing allele might have been caused by organophosphate resistance, the original reasons for its fast evolution and persistence must be related to some other phenotypic effect. To evaluate these possibilities, we need to understand the function of the truncated version of *CHKov1* and the phenotypic effects of the loss of its original function.

Recently, resistance to some *Bacillus thuringiensis* (Bt) toxins in *Heliothis virescens* was mapped to the TE-induced loss of Bt-toxin receptors (*15*). Moreover, resistance to dichlorodiphenyl-trichloroethane (DDT) in *D. melanogaster* (*16*) and possibly in *D. simulans* (*17*) is largely caused by the TE-induced up-regulation of a *cytp450* gene. Our research suggests another mechanism of pesticide resistance, which is mediated by either the loss of a nontarget gene or by the generation of a new protein. These cases underscore the importance of TEs to the evolution of pesticide resistance in particular and to adaptive evolution in general. The screen for adaptive TEs described in this work should help us understand the process and frequency of TE-generated adaptive mutations.

Table 1. The McDonald-Kreitman test of selection acting on *CHKov1*. Divergence is calculated in comparison with the sequence of *D. simulans*. The numbers in parentheses refer to the number of segregating sites in the haplotypes lacking *Doc1420*.

Gene region	Coding effect	Divergence	Polymorphism	*P* value
CHKov2	Synonymous	31	16 (13)	0.96 (0.80)
	Replacement	8	4 (4)	
CHKov1	Synonymous	36 (38)	12 (10)	0.03 (0.45)
	Replacement	19 (19)	17 (8)	
Transcript 1*	Synonymous	7	1	0.007
	Replacement	3	8	
Transcript 2*	Synonymous	16	5	0.66
	Replacement	9	4	

*Results for the positions within the first derived transcript or the second set of transcripts (assuming correct splicing of the third intron).

References and Notes

1. B. Charlesworth, C. H. Langley, *Annu. Rev. Genet.* **23**, 251 (1989).
2. J. S. Kaminker *et al.*, *Genome Biol.* **3**, 84 (2002).
3. E. Montgomery, B. Charlesworth, C. H. Langley, *Genet. Res.* **49**, 31 (1987).
4. D. A. Petrov, Y. T. Aminetzach, J. C. Davis, D. Bensasson, A. E. Hirsh, *Mol. Biol. Evol.* **20**, 880 (2003).
5. W. Bender, P. Spierer, D. S. Hogness, *J. Mol. Biol.* **168**, 17 (1983).
6. Materials and methods are available as supporting material on *Science* Online.
7. C. A. Russo, N. Takezaki, M. Nei, *Mol. Biol. Evol.* **12**, 391 (1995).
8. A. Bateman *et al.*, *Nucleic Acids Res.* **32**, 138D (2004).
9. J. Schultz, R. R. Copley, T. Doerks, C. P. Ponting, P. Bork, *Nucleic Acids Res.* **28**, 231 (2000).
10. M. N. Arbeitman *et al.*, *Science* **297**, 2270 (2002).
11. N. D. Singh, P. F. Arndt, D. A. Petrov, *Genetics* **169**, 709 (2005).
12. M. Slatkin, B. Rannala, *Annu. Rev. Genom. Hum. Genet.* **1**, 225 (2000).
13. J. H. McDonald, M. Kreitman, *Nature* **351**, 652 (1991).
14. N. D. Singh, D. A. Petrov, *Mol. Biol. Evol.* **21**, 670 (2004).
15. L. J. Gahan, F. Gould, D. G. Heckel, *Science* **293**, 857 (2001).
16. P. J. Daborn *et al.*, *Science* **297**, 2253 (2002).
17. T. A. Schlenke, D. J. Begun, *Proc. Natl. Acad. Sci. U.S.A.* **101**, 1626 (2004).

18. This work was supported by NSF grant 0317171 and by the Sloan Fellowship to D.A.P. J.M.M. is a Howard Hughes Medical Institute Predoctoral Fellow. The authors thank M. Feldman, S. Palumbi, P. Batterham, T. Wilson, L. Hadani, and members of D.A.P.'s lab for helpful discussions. We also thank T. Li and P. Carlson for help with experiments and J. Davis and O. Brandman for help with data analysis.

Supporting Online Material

www.sciencemag.org/cgi/content/full/309/5735/764/DC1
Materials and Methods
Figs. S1 and S2
Tables S1 to S3
References

24 March 2005; accepted 14 June 2005
10.1126/science.1112699

Regulation of X-Chromosome Counting by *Tsix* and *Xite* Sequences

Jeannie T. Lee*

In mammals, X-inactivation establishes X-chromosome dosage parity between males and females. How X-chromosome counting regulates this process remains elusive, because neither the hypothesized inactivation "blocking factor" nor the required cis-elements have been defined. Here, a mouse knockout and transgenic analysis identified DNA sequences within the noncoding *Tsix* and *Xite* genes as numerators. Homozygous deficiency of *Tsix* resulted in "chaotic choice" and a variable number of inactive X's, whereas overdosage of *Tsix*/*Xite* inhibited X-inactivation. Thus, counting was affected by specific *Tsix*/*Xite* mutations, suggesting that counting is genetically separable from but molecularly coupled to choice. The mutations affect XX and XY cells differently, demonstrating that counting and choice are regulated not by one "blocking factor," but by both a "blocking" and a "competence" factor.

During X-chromosome inactivation (XCI) (*1*), a "counting" mechanism ensures inactivation of only one X per diploid nucleus, and a "choice" mechanism randomly designates one active and one inactive X (X_a and X_i, respectively). Choice is known to be regulated by the noncoding genes, *Tsix* (*2*) and *Xite* (*3*), but how counting is regulated remains unknown. Specific X-linked "numerators" and autosomal "denominators" must participate, as counting depends on the X-to-autosome (X:A) ratio (*4*, *5*). Two models have been proposed. One postulates that a single "blocking factor," made up of a complex of X and autosomal factors, protects one X from inactivation and that supernumerary X's are silenced by default (*4*, *5*). A second model posits two factors: a blocking factor that protects the future X_a and an X-linked "competence factor" that induces XCI on the future X_i (*2*). The latter model requires purposeful—rather than default—action to achieve both the X_a and X_i.

A priori, any counting defect can be recognized by the appearance of an X_i in XY cells, absence of X_i in XX cells, or appearance of a second X_i in XX cells (Fig. 1A). Transgenic analyses in mouse embryonic stem (ES) cells reveal counting elements at the X-inactivation center (*Xic*), as XY cells display ectopic XCI in the presence of *Xic* transgenes (*6*–*8*). A 65-kb knockout (Δ65kb) (Fig. 1B) (*9*) suggests that numerators lie in a region that spans *Xist*, *Tsix*, *Xite*, *Tsx*, and *Chic1*, most likely within a 37-kb subregion (p37kb) (*10*). Yet, heterozygous deletions in the 5′ ends of

Howard Hughes Medical Institute, Department of Molecular Biology, Massachusetts General Hospital, Department of Genetics, Harvard Medical School Boston, MA 02114, USA.

*E-mail: lee@molbio.mgh.harvard.edu

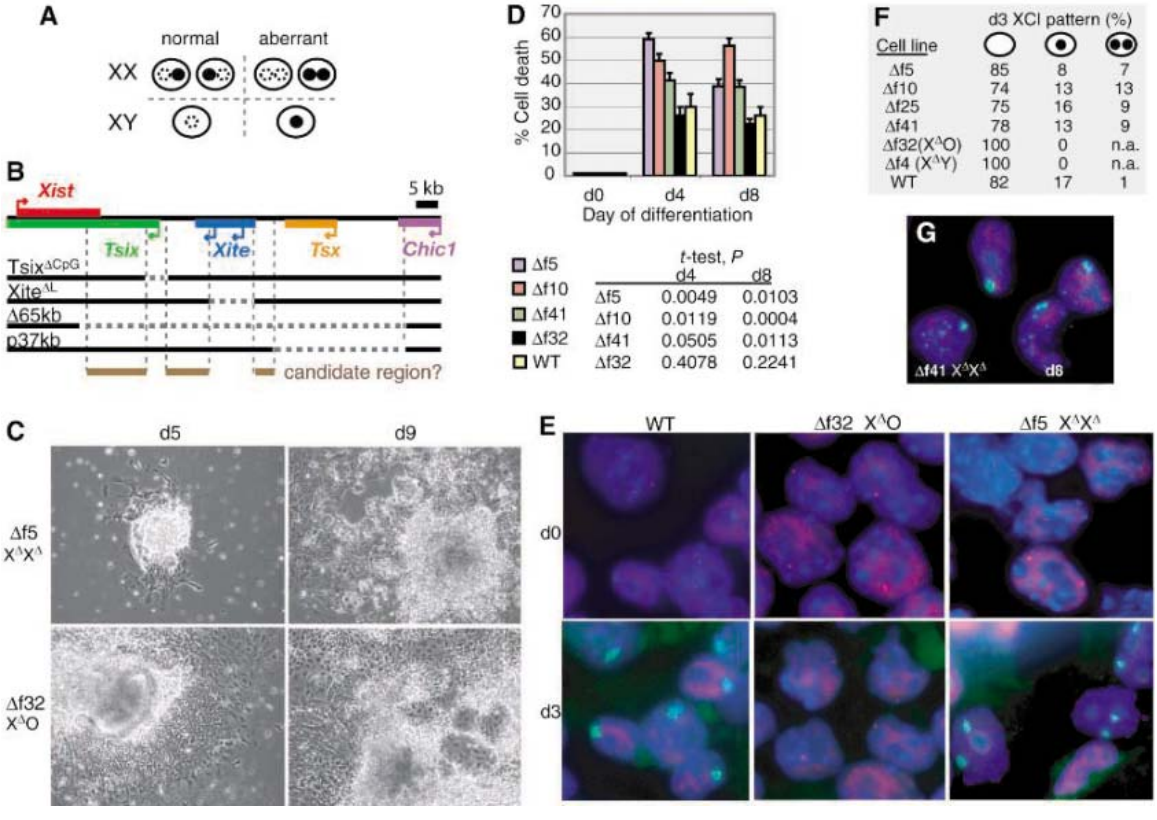


Fig. 1. Chaotic XCI in *Tsix* homozygous ES cells. (**A**) Normal and aberrant counting patterns. Solid circles, X_i; clear circles, X_a. (**B**) Existing *Xic* deletions suggest a tripartite counting domain (brown). Dotted lines delineate deletions. (**C**). Phase contrast images of mutant EB at the same magnification on the differentiation days (d) indicated. To generate EB, ES colonies were trypsinized into detached cellular clusters on d0, grown in suspension culture for 4 days in Dulbecco's modified Eagle's medium–15% fetal bovine serum without leukemia inhibitory factor, and adhered to gelatinized plates thereafter to obtain outgrowths (*2*). (**D**) Elevated cell death in $X^{\Delta}X^{\Delta}$ EB. Averages and standard deviations of three experiments are shown. For quantitation, cells in suspension and adherent EB were harvested on d0, d4, and d8 for trypan blue staining. The number of blue cells (dead) was divided by the total number of cells (blue + clear). The Student's *t* test was used to calculate statistical significance (*P*), with each mutant tested against the WT. (**E**) RNA/DNA FISH using probes for Xist RNA (labeled with fluorescein isothiocyanate, green) and Xite DNA (Cy3-labeled, red) to mark the X (*3*). Whole EB from d3 were dispersed and cytospun onto glass slides for analysis. (**F**) XCI patterns on d3 of differentiation. More than 200 nuclei from two experiments were counted. n.a., not applicable. (**G**) RNA/DNA FISH on d8 showed that surviving $X^{\Delta}X^{\Delta}$ cells (Δf41 shown) have one X_i. Xist RNA, green; Xite DNA, red.

Tsix and *Xite* do not affect counting, as Δ/Y males appropriately block XCI and Δ/+ females exhibit a single X_i (*2*, *3*) (*Tsix*$^{\Delta CpG}$ and *Xite*$^{\Delta L}$). Thus, counting elements may reside within a tripartite region downstream of *Xist*, excluding the 5′ ends of *Tsix* and *Xite* (Fig. 1B). However, recent observations in homozygous *Tsix* mutants ($X^{\Delta}X^{\Delta}$) raise new possibilities (*11*). Although they similarly lack *Tsix* function, $X^{\Delta}X^{\Delta}$ mice survive at only half the frequency of $X^{\Delta}Y$ littermates. Moreover, whereas $X^{\Delta}X$ mice preferentially inactivate the mutated X, surviving $X^{\Delta}X^{\Delta}$ animals revert to random XCI. Thus, in the presence of a second X, *Tsix* plays another role. Unmasked only when both alleles are deleted, this role has been proposed to be feedback regulation that ensures mutually exclusive choice of X_a and X_i, the loss of which results in "chaotic choice" and embryonic lethality when a large fraction of cells display aberrant XCI (*11*).

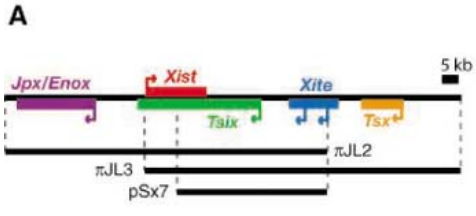
To test this model, I created an $X^{\Delta}X^{\Delta}$ ES model using blastocysts from an $X^{\Delta}X^{\Delta}$(neo^{-}) × X^{Δ}Y(neo^{-}) cross. From 93 blastocysts (11 crosses), 5 $X^{\Delta}X^{\Delta}$ ES lines were established (Δf1, Δf5, Δf10, Δf25, and Δf41), each of which carried two X's (fig. S1) and behaved similarly (table S1; fig. S2). X^{Δ}Y male (Δf4) and X^{Δ}O female (Δf21, Δf32) lines were isolated as controls. Whereas growth in the undifferentiated state revealed no differences between $X^{\Delta}X^{\Delta}$ and WT (wild-type) clones, differentiation into embryoid bodies (EB) uncovered clear differences. All differentiating $X^{\Delta}X^{\Delta}$ clones grew poorly as compared to WT, $X^{\Delta}X$, and X^{Δ}O controls, with $X^{\Delta}X^{\Delta}$ EB remaining small, tending to disintegrate during culture (Fig. 1C, fig. S2), and undergoing massive cell death between days 4 and 8 (Fig. 1D) (Student's *t* test, $P < 0.05$). Despite this result, a fraction of $X^{\Delta}X^{\Delta}$ EB did develop normal outgrowths with time (Fig. 1C, fig. S2), consistent with sporadic $X^{\Delta}X^{\Delta}$ mouse survival due to epigenetic differences among individual cells (*11*).

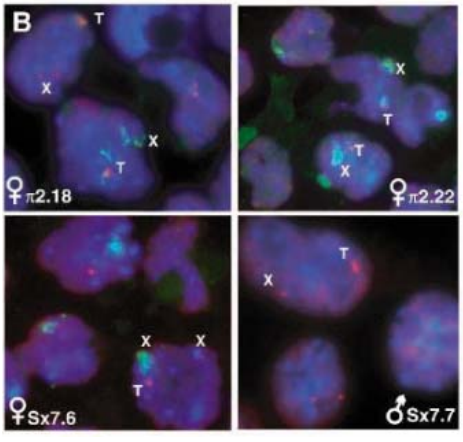
To monitor XCI, I performed RNA fluorescence in situ hybridization (FISH) to detect *Xist*-mediated silencing of the X_i (*12*, *13*). Although $X^{\Delta}X^{\Delta}$ clones appropriately maintained two X_a in the undifferentiated state (Fig. 1E), differentiating $X^{\Delta}X^{\Delta}$ cells displayed aberrant mixtures of cells with two X_a, one X_i, and two X_i, thus supporting the idea of chaotic choice (Fig. 1, E and F) (cells with two X_a had either failed to undergo XCI or had not yet done so). Robust EB were dominated by cells with only one X_i (Fig. 1G), whereas runted EB showed a higher proportion of cells with two X_i. WT and $X^{\Delta}X$ cells did not exhibit two X_i on any day of differentiation. Moreover, X^{Δ}O and X^{Δ}Y cells did not exhibit aberrant XCI, despite also lacking *Tsix* (Fig. 1, E and F). These data indicated that the $X^{\Delta}X^{\Delta}$ phenotype did not arise simply from absent *Tsix* function nor sex per se; instead, it required both an XX and a *Tsix*$^{-/-}$ background. Thus, a second X^{Δ} indeed unmasked further function for *Tsix*.

What was this function? In $X^{\Delta}X^{\Delta}$ cells, the deviation from the expected X_i number suggested a counting defect (Fig. 1A). To test *Tsix*'s potential role as a counting element, I introduced supernumerary copies into WT ES cells and examined whether XCI patterns were altered. In XY cells, multicopy *Xic* transgenes lead to ectopic inactivation of the X or the transgene-bearing autosome in cis (*6*–*8*). Because of potential XX-XY differences, I recreated the transgenics in XX cells using the 80-kb πJL2 and πJL3 *Xic* transgenes (Fig. 2A) (*8*). Three representative lines with low and high transgene copy numbers (fig. S3) were characterized in detail.

FISH analysis revealed Xist RNA accumulation on either the X's or transgene-bearing autosome (Fig. 2, B and C), consistent with results in XY cells (*8*). However, whereas XY lines differentiated well (*8*), XX lines showed aberrant EB outgrowth as compared to vector-only WTneo controls (Fig. 2D, fig. S4). A potential complication, however, was the presence of *Xist* in πJL2 and πJL3, which could affect EB growth through autosomal inactivation (*8*). To exclude this possibility, I generated clones from the *Xist*-deficient transgene, pSx7 (Fig. 2A). pSx7 clones also demonstrated XX-XY differences. In contrast to XY lines, XX lines yielded little EB outgrowth (Fig. 2D, fig. S4), thereby confirming an XX-specific defect due to overdosage of *Xic* sequences.

To pinpoint specific numerators, I tested subfragments of pSx7 (Fig. 3A). From tests of four independent clones per subfragment, XX clones grew similarly to XX controls and XY transgenic clones before differentiation, but differentiation again elicited marked XX-XY differences (Fig. 3, B and C; fig. S4, table S1). The disparities were most profound for p3.7 and pXite, but pCC3, pCC4, and pSxn also showed significant growth differences (fig. S4; table S1). During suspension culture on days 0 to 4, XX mutants exhibited unusually fast radial growth. During adherent culture on days 5 and 6, they continued to grow radially without obvious differentiation, as shown by their





C

♀	Tg copy number	Xist RNA domains on day 4 (%) 0	1X	2X	Tg	X + Tg
π 2.1B	low	77	1	0	21	1
π 2.18	high	86	1	0	11	2
π 2.22	low	56	31	0	3	10
π 3.2B	high	88	12	<<1	0	0
π 3.10	low	70	30	0	0	0
π 3.15	high	99	1	0	0	0
Sx7.1B	low	72	28	0	0	0
Sx7.4	high	99	1	<<1	0	0
Sx7.6	low	89	10	1	0	0
WTneo	n.a.	73	27	0	n.a.	n.a.

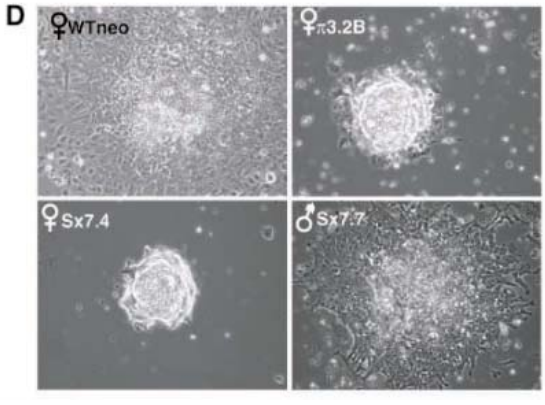


Fig. 2. Transgenic XX ES lines carrying the *Xic*. (**A**) Map of P1 *Xic* transgenes. πJL2 (80 kb) contains *Xist* and 30 kb of upstream and downstream sequence; πJL3 (80 kb) contains *Xist* and 60 kb of downstream sequence; pSx7 contains the Bss HII–Not I fragment of πJL1 (*8*). Transgenes were introduced by electroporation (*8*) together with a Neo-marker (pGKRN) at 0.1 M ratio. All transgenes were inserted into autosomes. (**B**) RNA/DNA FISH detected Xist RNA (green) and the X (Xite DNA, red) in d4 EB. T, transgene-bearing autosome. (**C**) XCI patterns in d4 transgenic XX EB. Tg, transgene. (**D**) Phase contrast images of WT and transgenic EB on d5 at the same magnification.

smoothly round appearance and lack of cellular outgrowth (Fig. 3C). After day 7, however, XX mutants underwent massive cell death and disintegrated (Fig. 3, B and C). None of the WTneo XX controls and XY lines carrying the same transgenes showed these effects. Neither did any of the XX lines carrying sequences from *Xist* or *Tsx*. Thus, the mutant phenotype was XX-specific and specific to the 5′ ends of *Tsix* and *Xite*.

RNA/DNA FISH showed a total failure of *Xist* up-regulation in the *Tsix*/*Xite* transgenic XX lines from day 2 until EB degeneration (Fig. 3, D and E, table S1) (0%, n > 50,000), indicating that the growth abnormalities resulted from arrest of XCI. This result contrasted with the occurrence of Xist$^+$ cells in the πJL2, πJL3, and pSx7 transgenic XX series

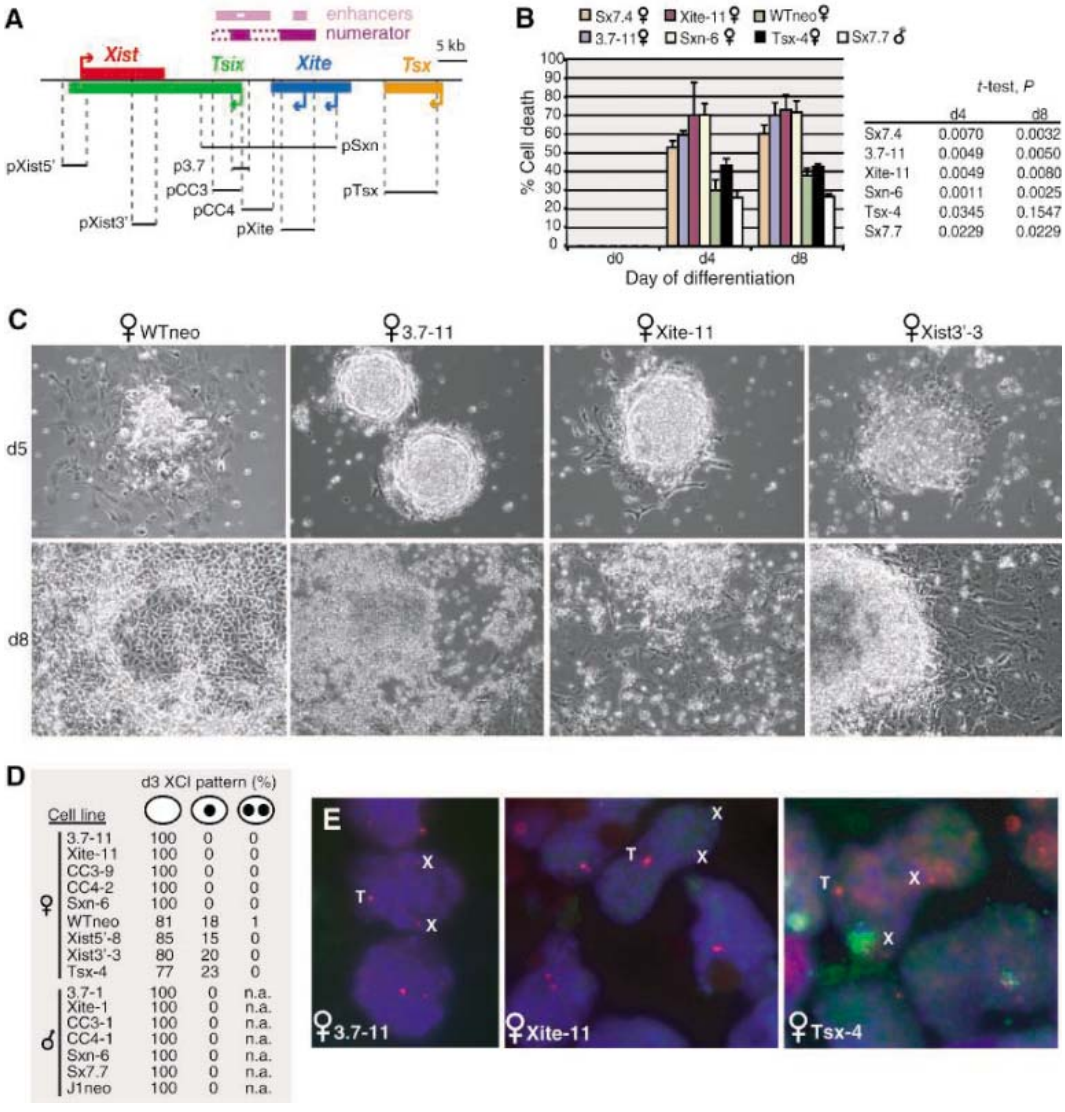


Fig. 3. *Tsix* and *Xite* contain numerators. (**A**) Fragmentation of *Xic* transgenes. pSxn, a 19.5-kb Rsr II–Not I fragment of πJL1; p3.7, the 3.7-kb Mlu I–Sac I sequence deleted from *Tsix*$^{\Delta CpG}$ (*2*); pCC3, a 4.3-kb Bam HI fragment downstream of *Tsix*'s start; pCC4, a 5.9-kb Bam HI fragment including part of the *Tsix* promoter; pXite, a 5.6-kb fragment spanning *Xite*'s DNase I hypersensitive sites DHS1 to DHS4 and carrying the 1.2-kb *Tsix* enhancer (*3*, *14*); pXist5′, a 4.8-kb Xba I–Xho I fragment from the *Xist* promoter; pXist3′, a 4.9-kb Pst I fragment from *Xist* exon 7; and pTsx, GenBank X99946 bp 41,347 to 52,236 from *Tsx*. All transgenes except pXist3′ contain Neo. All lines have autosomal insertions. Solid dark purple bars, region with strongest counting phenotype; dashed purple bars, additional numerator elements. (**B**) Cell death analysis by the trypan blue assay as detailed in Fig. 1D. To calculate *P*, mutants were tested against WTneo. (**C**) Phase contrast EB images at the same magnification. (**D**) XCI patterns on d3. Between 200 and 50,000 nuclei from two experiments were examined. EB were trypsinized and cytospun onto glass slides. n.a., not applicable. (**E**) RNA/DNA FISH detects Xist RNA (green) and the transgene-bearing autosome using p3.7, pXite, or pTsx plasmids as probe (red). T, transgenic autosome.

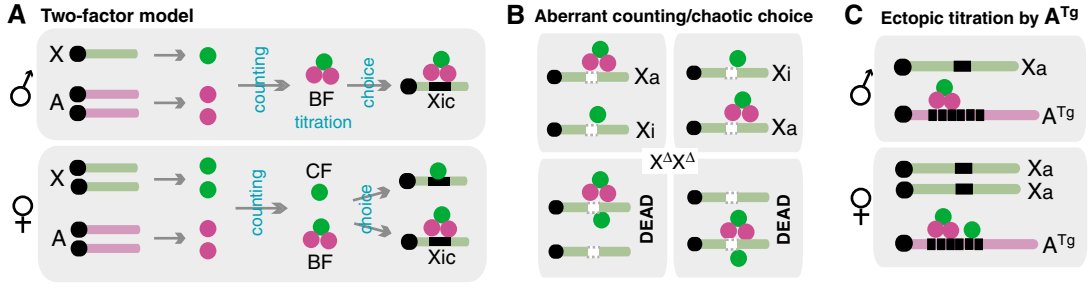


Fig. 4. The two-factor model for counting. (**A**) In the two-factor model, "counting" represents the titration of A- and X-factors to form BF. Untitrated X-factor(s) becomes CF. X-factors can be a diffusible factor or a cis-element. "Choice" reflects BF binding to *Tsix* on the future X_a and CF binding to induce *Xist* on the future X_i. BF and CF bind mutually exclusively (mediated by *Tsix*) to the 15-kb numerator. (**B**) The two-factor model reconciles aberrant counting and chaotic choice in $X^{\Delta}X^{\Delta}$. Depicted are four equally likely outcomes in $X^{\Delta}X^{\Delta}$ undergoing XCI. The top outcomes yield overtly normal XCI and viable cells. The bottom outcomes represent loss of mutual exclusion and inviable states with $2X_i$ or $2X_a$. Chromosome gap, *Tsix*$^{\Delta CpG}$. (**C**) The two-factor model also reconciles failure of XCI in transgenic XX cells. Multicopy *Tsix*/*Xite* transgenes on autosomes outcompete X's for BF and CF, thereby precluding XCI in XX cells. XY transgenic cells lack CF and are therefore protected from XCI. Black boxes, *Tsix*/*Xite* transgenes. A^{Tg}, transgene-bearing autosome.

and suggested that elements necessary for *Xist* induction were absent in the smaller transgenes. The absence of XCI was not due to XO aneuploidy, as DNA FISH demonstrated an XX constitution (Fig. 3E). None of the XY *Tsix/Xite* lines nor any of the XX lines made from *Xist*, *Tsx*, or vector-only transgenes showed aberrant XCI patterns, consistent with their normal growth. Therefore, failure of XCI and arrested ES cell differentiation resulted specifically from overdosage of 5′ *Tsix/Xite* sequences in an XX background.

Thus, homozygosing $Tsix^{\Delta CpG}$ and overdosing *Tsix/Xite* produced opposite phenotypes in XX cells: Whereas $Tsix^{-/-}$ cells chaotically inactivated 0, 1, or 2 X's, cells with supernumerary *Tsix/Xite* copies failed to initiate XCI. The 15 kb encompassing the 5′ ends of *Tsix* and *Xite* must therefore be dosage-sensitive, requiring precise titration for initiation of XCI. I propose that the 15-kb sequence functions as an X-linked numerator and that subtracting or adding this sequence alters the X:A ratio. Interestingly, this region contains two *Tsix*-specific enhancers (Fig. 3A) (*14*). The idea that *Tsix* enhancers are numerators is inherently satisfying, as the fate of each X is ultimately determined by whether *Tsix* expression persists (X_a) or is switched off (X_i). Given that the strongest effects occurred with p3.7 and pXite, two fragments that contain promoter activity (*14*), the effects could be transcription- or RNA-mediated. Although truncated transcripts could be detected by RNA FISH, the numerator more likely acts as a titratable DNA sequence because promoter-deficient transgenes, such as pCC3 and pCC4, also elicited a phenotype.

How do these conclusions affect proposed counting models? In the single–blocking factor model (fig. S5) (*4*, *5*), the X and a subset of autosomes (A) produce specific factors in quantities proportional to their copy number. The complex of X- and A-factors forms the blocking factor (BF), which then blocks the firing of one *Xic* per cell. All remaining X's are inactivated by default. However, this model cannot explain the absence of XCI in *Tsix/Xite* XX transgenics, as the occurrence of only one BF should allow default silencing of the second X. Nor can it explain the mixture of *Tsix* $X^{\Delta}X^{\Delta}$ cells with one or two X_i. If BF binds outside of the deleted region, only one X_i should ever be observed. If BF binds within the deleted region, two X_i should always be observed. Furthermore, given that *Tsix* represses *Xist* in XX cells, the absence of XCI in $X^{\Delta}Y$ cells seems incongruous (*2*). Finally, the model cannot reconcile the differential XX-XY response to *Tsix/Xite* overdosage.

These arguments therefore appeal to a two-factor model (Fig. 4A, fig. S5) (*2*), in which a repressive factor (BF) designates the X_a but a second, inductive factor must also be present to inactive the remaining X. During counting, the titration of X- and A-factors produces BF, and any untitrated X-factor becomes the "competence factor" (CF). "Choice" then reflects binding of BF to the future X_a and CF to the future X_i. Through joint requirements for *Tsix*/*Xite*, counting and choice are molecularly coupled, albeit genetically separable [*Tsix* heterozygotes lose random choice but preserve counting (*2*)]. The current work suggests that *Tsix* mediates the mutually exclusive binding of BF and CF. In $X^{\Delta}X^{\Delta}$ mutants, chaotic choice arises from lack of mutual exclusion, thereby producing cells with $0X_i$, $1X_i$, or $2X_i$ (Fig. 4B) (*11*). By definition and as implied by the data, BF and CF sites must lie within the 15-kb numerator, likely within the *Tsix* enhancers. In XX transgenics, multiple ectopic copies of *Tsix* and *Xite* titrate away BF and CF, thereby precluding XCI (Fig. 4C). High transgene copy numbers render autosomes more competitive for BF and CF than the Xs, explaining why an X_i is rarely, if ever, seen. The absence of any X_i may suggest cooperative interactions among tandem transgenes. By this model, XY transgenics and *Tsix* $X^{\Delta}Y$ cells suffer no consequence, because the absence of CF precludes XCI. In sum, this work underscores the importance of comparing homo- and heterozygous mutants in epigenetic phenomena. It also raises new questions about how counting takes place (SOM Text). By identifying *Tsix* and *Xite* as numerators and establishing a two-factor model, the study provides a conceptual framework for further dissection of the counting mechanism.

References and Notes

1. M. F. Lyon, *Nature* **190**, 372 (1961).
2. J. T. Lee, N. Lu, *Cell* **99**, 47 (1999).
3. Y. Ogawa, J. T. Lee, *Mol. Cell* **11**, 731 (2003).
4. M. F. Lyon, *Biol. Rev. Camb. Philos. Soc.* **47**, 1 (1972).
5. S. Rastan, *J. Embryol. Exp. Morphol.* **78**, 1 (1983).
6. J. T. Lee, W. M. Strauss, J. A. Dausman, R. Jaenisch, *Cell* **86**, 83 (1996).
7. E. Heard, F. Mongelard, D. Arnaud, P. Avner, *Mol. Cell. Biol.* **19**, 3156 (1999).
8. J. T. Lee, N. F. Lu, Y. Han, *Proc. Natl. Acad. Sci. U.S.A.* **96**, 3836 (1999).
9. P. Clerc, P. Avner, *Nat. Genet.* **19**, 249 (1998).
10. C. Morey *et al.*, *EMBO J.* **23**, 594 (2004).
11. J. T. Lee, *Nat. Genet.* **32**, 195 (2002).
12. C. J. Brown *et al.*, *Cell* **71**, 527 (1992).
13. G. D. Penny, G. F. Kay, S. A. Sheardown, S. Rastan, N. Brockdorff, *Nature* **379**, 131 (1996).
14. N. Stavropoulos, R. K. Rowntree, J. T. Lee, *Mol. Cell. Biol.* **25**, 2757 (2005).

15. I thank Y. Ogawa for pSx7 and pXite (pDNT1); N. Xu for pTsx; L. F. Zhang, D. E. Cohen, N. Xu, B. K. Sun, R. J. Spencer, D. Tian, and J. E. Kirby for critical reading; and all lab members for stimulating discussion. J.T.L. is an Investigator of the Howard Hughes Medical Institute and is supported by the NIH (RO1-GM58839).

Supporting Online Material
www.sciencemag.org/cgi/content/full/309/5735/768/DC1
SOM Text
Figs. S1 to S5
Table S1
References and Notes

18 April 2005; accepted 9 June 2005
10.1126/science.1113673

Organization of Iron-Sulfur Clusters in Respiratory Complex I

Philip Hinchliffe and Leonid A. Sazanov*

Complex I of respiratory chains plays a central role in bioenergetics and is implicated in many human neurodegenerative diseases. An understanding of its mechanism requires a knowledge of the organization of redox centers. The arrangement of iron-sulfur clusters in the hydrophilic domain of complex I from *Thermus thermophilus* has been determined with the use of x-ray crystallography. One binuclear and six tetranuclear clusters are arranged, maximally 14 angstroms apart, in an 84-angstrom-long electron transfer chain. The binuclear cluster N1a and the tetranuclear cluster N7 are not in this pathway. Cluster N1a may play a role in the prevention of oxidative damage. The structure provides a framework for the interpretation of the large amounts of data accumulated on complex I.

Nicotinamide adenine dinucleotide (NADH): ubiquinone oxidoreductase (complex I, EC 1.6.5.3) is the first enzyme of the mitochondrial and bacterial respiratory chains. It catalyzes the transfer of two electrons from NADH to quinone, coupled to the translocation of about four protons across the membrane (*1*, *2*). This process accounts for about 40% of the transmembrane proton gradient generated in NADH oxidation by the mitochondrial respiratory chain. Complex I is one of the largest membrane protein complexes known to date. The simplest version is the prokaryotic enzyme, which has 14 subunits with a combined molecular mass of about 550 kD. Analogs of all of the subunits of bacterial complex I (also referred to as NDH-1) are found in the more elaborate mitochondrial enzyme, and they both contain equivalent redox components (*2*). Mitochondrial and bacterial enzymes have a

Medical Research Council (MRC) Dunn Human Nutrition Unit, Wellcome Trust/MRC Building, Hills Road, Cambridge CB2 2XY, UK.

*To whom correspondence should be addressed. E-mail: sazanov@mrc-dunn.cam.ac.uk

characteristic L-shaped structure with one arm (hydrophobic) embedded in the membrane and the other, the hydrophilic peripheral arm, protruding into the mitochondrial matrix or the bacterial cytoplasm (*3*–*7*). These similarities allow NDH-1 to be used as a minimal model for the study of complex I. Its atomic structure is not known, and the mechanisms of electron transfer and proton pumping are not established. The association of mutations in complex I subunits with human neurodegenerative diseases, including Parkinson's disease (*8*), provides additional impetus to efforts to characterize this enzyme. Complex I has also been suggested to be a major source of reactive oxygen species (ROS) production in mitochondria, which can lead to mitochondrial DNA damage and may be one of the causes of aging (*9*). Dissociation of complex I with chaotropes and detergents has demonstrated that its NADH-binding site and all of its redox centers [noncovalently bound flavin mononucleotide (FMN) and iron-sulfur clusters] are in the peripheral arm (*1*, *10*, *11*), whereas the proton-pumping machinery is probably in the membrane arm. On the basis of such fragmentation studies, we have recently proposed a detailed model of the arrangement of subunits in bacterial complex I (*12*). It is broadly consistent with models developed from consideration of the evolutionary origins of complex I from smaller preexisting modules, such as soluble and membrane-bound hydrogenases (*1*, *13*). Subunits Nqo1 to Nqo3 (*Thermus* nomenclature is used throughout, with bovine mitochondrial nomenclature shown in parentheses when needed for clarity) form the dehydrogenase domain, capable of NADH oxidation with artificial electron acceptors. It is at the distal end of the peripheral arm. Subunits Nqo4 to Nqo6 and Nqo9 are proposed to form a connecting domain between the dehydrogenase domain and the membrane arm (*14*). Iron-sulfur (Fe-S) clusters identified with the use of electron paramagnetic resonance spectroscopy (EPR) include the binuclear clusters N1a and N1b and the tetranuclear clusters N2, N3, N4, and N5 (*2*, *15*). Sequence comparisons suggest that complex I contains two more tetranuclear clusters, N6a and N6b, in subunit Nqo9 (TYKY). So far, they have been detected only in subcomplexes or in the recombinant subunit (*16*, *17*). Additionally, complex I from *Escherichia coli*, from *Thermus thermophilus*, and from various other bacteria contains another binding motif for the tetranuclear cluster N7, which has been identified by EPR in recombinant subunit Nqo3 (75 kD) but not in situ (*18*). Thus, complex I is predicted to contain a total of eight or nine Fe-S clusters, making it the most elaborate iron-sulfur protein assembly known.

We have purified and crystallized the hydrophilic domain (peripheral arm) of complex I from *T. thermophilus* HB-8 (*19*). It contains all the redox centers and represents more than half of the molecular mass of the entire complex (280 kD out of 520 kD). Analysis of x-ray data using anomalous Fe signals from intrinsic Fe-S clusters allowed us to identify nine Fe-S clusters in this subcomplex and calculate electron density at about 4 Å resolution (table S1). The overall shape of the hydrophilic domain was clear, and many secondary structure features, such as α helices, were visible (fig. S1). The molecular surface of the peripheral arm of complex I and the electron densities of Fe-S clusters are shown in Fig. 1. The clusters are buried within the protein mass, shielded from the solvent phase. The overall appearance of the peripheral arm is pronouncedly Y-shaped, the feature which is partially visible in electron microscopic models of complex I from *Neurospora crassa* (*4*) and from the hyperthermophile *Aquifex aeolicus* (*6*). The domain is about 155 Å high with an average width of about 70 to 80 Å. These dimensions and overall shape are consistent with those of the peripheral arm in the enzyme from *A. aeolicus*, indicating that the lower part of the structure, as shown in Fig. 1, forms the interface with the membrane arm of the enzyme.

From an examination of the electron density of Fe-S clusters, it is clear that two of the clusters are oblong and smaller than the seven other clusters (Fig. 1). Accordingly, during heavy atom refinement [where the clusters were treated as single superatoms (*19*)], the occupancies of the two smaller clusters were always about half the occupancies of the seven larger clusters. Thus, the two smaller clusters have been assigned as being binuclear and the seven larger ones as tetranuclear. The numbers of the two cluster types are consistent with the maximum number predicted from EPR experiments and sequence comparisons, indicating that our model for the arrangement of clusters in complex I is complete. Mitochondrial complex I and many bacterial enzymes do not contain binding motifs for cluster N7, and so they will contain the two binuclear clusters and the six tetranuclear clusters.

The detailed arrangement of iron-sulfur clusters is shown in Fig. 2. Seven of them are arranged in a continuous chain (indicated by arrows in Fig. 2) with edge-to-edge spacing within 14 Å, the maximum distance for physiological electron transfer reactions (*20*). This chain, about 84 Å long, is likely to connect the two catalytic sites of the enzyme. It terminates at the tetranuclear cluster close to the interface with the membrane arm. This cluster is likely to be N2, which has the highest (about –100 mV on average), pH-dependent, midpoint potential. It is in the connecting domain subunit Nqo6 (PSST), and it has been suggested that it reduces the quinone at the interface with the membrane domain (*1*, *2*). Cluster N2 is within about 15 Å of the membrane end of the hydrophilic domain and appears to be next to a short channel in which

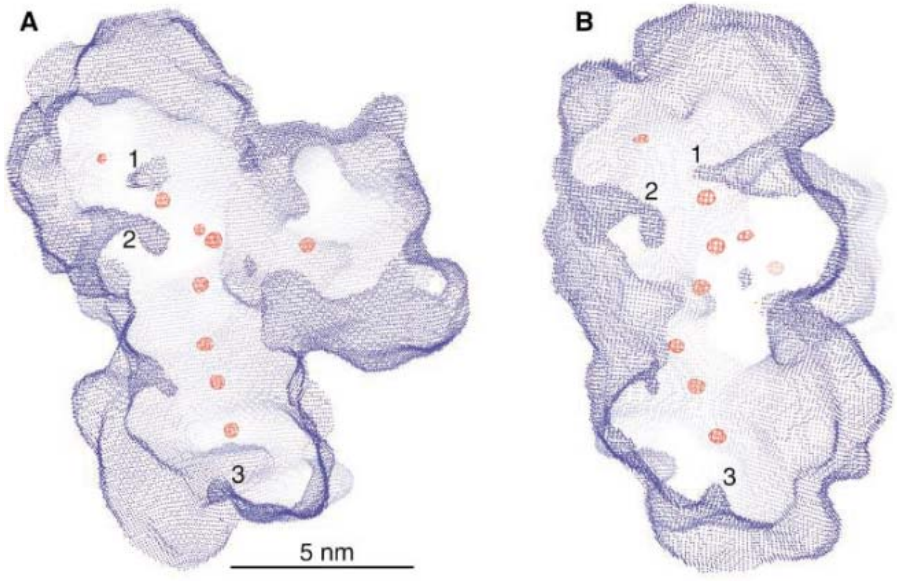


Fig. 1. The electron density of Fe-S clusters shown within the molecular surface of the peripheral arm of complex I from *T. thermophilus*. To calculate the molecular surface, a mask was produced with the CCP4 program Ncsmask (*30*), using skeletonized density from one hydrophilic domain and a radius of 5 Å around skeleton atoms. The clusters are shown in red as the electron density contoured at 6σ. The number 1 denotes a possible channel for NADH access, reaching within 10 Å of the first Fe-S cluster in the redox chain (N3); 2 denotes a second possible access channel, approaching within about 15 Å of the same cluster; and 3 denotes a short channel that would allow quinone to approach the last cluster in the chain of Fe-S clusters (N2). (**A**) The Y-shaped view of the domain, with the scale bar shown. The interface with the membrane domain is at the bottom of the structure. (**B**) Orthogonal view, rotated clockwise about the vertical compared with (A).

the electron acceptor could sit within about 10 Å of N2 (Fig. 1 and fig. S1). This is in contrast to the suggestion that cluster N2 is about in the middle of the peripheral arm and that ubiquinone moves inside a long channel to reach this cluster (*3*). Our interpretation is consistent with the observation that a semiquinone species forms within 12 Å of cluster N2 (*21*). Although a complete Q binding site will only be revealed in a structure of intact enzyme, it is clear now that the quinone can reside mostly within the membrane, as expected for such a hydrophobic moiety.

All remaining clusters can be assigned tentatively on the basis of current knowledge about the arrangement of subunits in complex I and about the association of particular clusters with specific subunits (*2*, *12*, *15*). This assignment can be verified once an atomic model is built and refined. The binuclear cluster at the top left in Fig. 2 and the tetranuclear cluster at the top right are separated from the other clusters by about 19 Å and 21 Å edge-to-edge distances, respectively, and so they are unlikely to participate directly in physiological electron transfer. The two tetranuclear clusters above N2 and immediately preceding it in the electron transfer chain are likely to be N6a and N6b, because they are coordinated by the ferredoxin-like subunit Nqo9, thought to be in the connecting domain, close to the interface with the membrane arm (*2*, *17*). Accordingly, the relatively large cluster-free domain to the right of clusters N2 and N6 (Fig. 1B) is likely to consist of the remaining subunits of the connecting domain, Nqo4 (49 kD) and Nqo5 (30 kD).

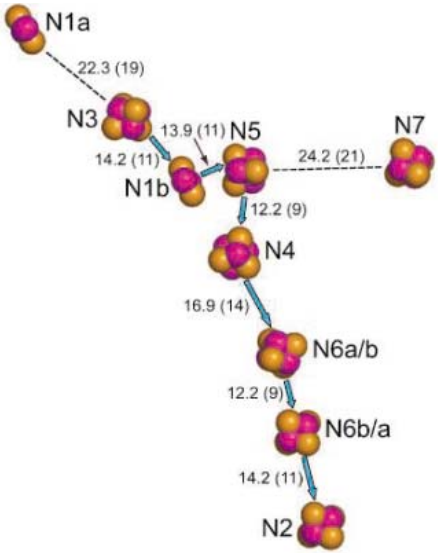


Fig. 2. Arrangement of Fe-S clusters in complex I. Metal sites are composed of magenta spheres for Fe atoms and yellow spheres for S atoms. The overall orientation is similar to the one shown in Fig. 1A. Most of the Fe-S clusters are roughly in the plane of the Y, with cluster N1b offset toward the reader by about 14 Å. Cluster names have been assigned tentatively as described in the text. Cluster N1a is located within subunit Nqo2; N3 in Nqo1; N1b, N4, N5, and N7 in Nqo3; N6a/b in Nqo9; and N2 in Nqo6. The likely pathway of electron transport is indicated by blue arrows. The distances between the clusters given in Å were calculated both center to center and edge to edge (shown in parentheses). Clusters N3 and N5 are separated by 17.6 Å (about 15 Å edge to edge), and clusters N1b and N4 by 19.2 Å (about 16 Å edge to edge).

Subunit Nqo1 (51 kD) contains the NADH-binding site, the primary electron acceptor FMN (midpoint potential –340 mV), and the tetranuclear cluster N3. Therefore, the first tetranuclear cluster in the chain of Fe-S clusters is likely to be N3 (Fig. 2), because subunits Nqo1 and Nqo2 (24 kD) can form a distinct subcomplex capable of NADH oxidation (*2*, *22*) and Nqo2 contains only the binuclear cluster, N1a. This proposal is consistent with the observed strong spin-spin interaction between the semiflavin and cluster N3 (*15*). The first cluster in the chain of Fe-S clusters should be close to the NADH-binding site. Accordingly, there appear to be two channels in this region of the electron density map, each of which can connect the bulk of solvent phase to about 10 to 15 Å from the cluster N3 (Fig. 1).

Subunit Nqo3 contains the binuclear cluster N1b as well as tetranuclear clusters N4, N5, and N7 (*18*). Cluster N7 is not present in many species and so may not be a part of the conserved electron transfer pathway. Therefore, it is the most plausible candidate for the distal tetranuclear cluster (Fig. 2). This location is in contrast to the proposal that cluster N7 has a role in electron transfer to the low-potential bacterial electron acceptor menaquinone (*23*). Rather, the role of N7 may be to stabilize the fold of the Nqo3 subunit, which has no other clusters in its extensive C-terminal domain. This arrangement indicates that the three remaining clusters between N3 and N7 are N1b, N4, and N5. This suggestion provides a reasonably compact arrangement within subunit Nqo3, because clusters N1b, N4, and N5 are coordinated within a segment of about 220 residues in the N-terminal domain of Nqo3. Thus, the binuclear cluster in this group is proposed to be N1b, whereas cluster N5 rather than cluster N4 is likely to be in its assigned position because of its unusual EPR properties: It exhibits very fast spin relaxation and it appears to exist in a mixed spin ground state ($S = 1/2 + 3/2$) (*24*). Unlike the other clusters shown in Fig. 2, N5 in its proposed position can interact with more than two clusters (namely with N3, N1b, and N4), which may account for its unusual EPR signals.

The distance between N3 and N5 is greater than the distance between N3 and N1b. Thus, electron transfer from FMN to quinone probably takes the shortest route along the following path: N3-N1b-N5-N4-N6a/b-N6b/a-N2, as indicated by the arrows in Fig. 2. Clusters N1b, N3, N4, and N5 are isopotential (about –250 mV) in situ (*15*).

This arrangement leaves the binuclear cluster N1a as the only possible candidate for its assigned position, which is also consistent with the co-localization of subunits Nqo1 and Nqo2. Because of its large distance from N3, it is unlikely to participate directly in the electron transfer from FMN to the quinone. This may explain why cluster N1a is not reduced by NADH in most species and has the lowest midpoint potential (about –370 mV on average) (*15*). Judging from the positions of possible NADH access channels in the electron density (Fig. 1), it is likely that FMN is located between N1a and N3, within 14 Å from either cluster. In this case, one role for the N1a cluster, because of its low potential, could be to interact with FMN and prevent its excessive reduction by redistribution of electrons further down the redox chain. This mechanism could prevent excessive generation of damaging ROS by the reduced flavin, which is likely to be more exposed to the solvent than the Fe-S clusters. A similar role was suggested for heme *b* in succinate dehydrogenase (*25*). It would appear that instead of adding a high-potential cofactor near the end of the chain, as is the case for succinate dehydrogenase, in complex I nature has opted for adding a very low–potential cofactor near the beginning of the chain, with a similar effect on the reduction state of the flavin. It will be of great interest to explore, by genetic modification, how changes to the environment of cluster N1a affect the rates of electron transfer and of ROS generation.

The organization of Fe-S clusters in complex I determined here allows us to start to understand the electron transfer mechanism of this enormously complicated enzyme, and it provides a framework for interpreting the vast amount of data accumulated over the past 40 years. It is clear that the fundamental design of the hydrophilic part of complex I is similar to other electron transfer proteins: two catalytic sites are connected by a redox chain (*26*). However, the chain does not take the simplest, shortest route between the two catalytic sites. In principle, fast electron transfer could be provided with just one cluster in place of N1b and N5. The reason for this added complexity may be related either to the evolutionary origins of complex I from smaller building blocks or to its mechanism. The coupling between electron transfer and proton translocation may be either direct (e.g., Q cycle) or indirect (conformational) (*1*, *2*, *27*–*29*). The arrangement of clusters described here is compatible with a direct mechanism, but it does not exclude an indirect coupling or a combination of both mechanisms.

References and Notes

1. J. E. Walker, *Q. Rev. Biophys.* **25**, 253 (1992).
2. T. Yagi, A. Matsuno-Yagi, *Biochemistry* **42**, 2266 (2003).
3. V. Zickermann *et al.*, *J. Biol. Chem.* **278**, 29072 (2003).
4. V. Guenebaut, R. Vincentelli, D. Mills, H. Weiss, K. R. Leonard, *J. Mol. Biol.* **265**, 409 (1997).
5. N. Grigorieff, *J. Mol. Biol.* **277**, 1033 (1998).
6. G. Peng *et al.*, *Biochemistry* **42**, 3032 (2003).
7. V. Guenebaut, A. Schlitt, H. Weiss, K. Leonard, T. Friedrich, *J. Mol. Biol.* **276**, 105 (1998).
8. A. H. Schapira, *Biochim. Biophys. Acta* **1364**, 261 (1998).
9. R. S. Balaban, S. Nemoto, T. Finkel, *Cell* **120**, 483 (2005).
10. T. Friedrich, *Biochim. Biophys. Acta* **1364**, 134 (1998).
11. L. A. Sazanov, S. Y. Peak-Chew, I. M. Fearnley, J. E. Walker, *Biochemistry* **39**, 7229 (2000).
12. P. J. Holt, D. J. Morgan, L. A. Sazanov, *J. Biol. Chem.* **278**, 43114 (2003).
13. T. Friedrich, D. Scheide, *FEBS Lett.* **479**, 1 (2000).
14. H. Leif, V. D. Sled, T. Ohnishi, H. Weiss, T. Friedrich, *Eur. J. Biochem.* **230**, 538 (1995).
15. T. Ohnishi, *Biochim. Biophys. Acta* **1364**, 186 (1998).
16. T. Yano, S. Magnitsky, V. D. Sled, T. Ohnishi, T. Yagi, *J. Biol. Chem.* **274**, 28598 (1999).
17. T. Rasmussen *et al.*, *Biochemistry* **40**, 6124 (2001).
18. E. Nakamaru-Ogiso, T. Yano, T. Yagi, T. Ohnishi, *J. Biol. Chem.* **280**, 301 (2005).
19. Materials and methods are available as supporting material on *Science* Online.
20. C. C. Page, C. C. Moser, X. Chen, P. L. Dutton, *Nature* **402**, 47 (1999).
21. T. Yano, W. R. Dunham, T. Ohnishi, *Biochemistry* **44**, 1744 (2005).
22. T. Yano, V. D. Sled, T. Ohnishi, T. Yagi, *J. Biol. Chem.* **271**, 5907 (1996).
23. M. Finel, *Biochim. Biophys. Acta* **1364**, 112 (1998).
24. T. Yano *et al.*, *J. Biol. Chem.* **278**, 15514 (2003).
25. V. Yankovskaya *et al.*, *Science* **299**, 700 (2003).
26. C. C. Page, C. C. Moser, P. L. Dutton, *Curr. Opin. Chem. Biol.* **7**, 551 (2003).
27. A. A. Mamedova, P. J. Holt, J. Carroll, L. A. Sazanov, *J. Biol. Chem.* **279**, 23830 (2004).
28. T. Friedrich, *J. Bioenerg. Biomembr.* **33**, 169 (2001).
29. G. Belogrudov, Y. Hatefi, *Biochemistry* **33**, 4571 (1994).
30. Collaborative Computational Project Number 4, *Acta Crystallogr.* **D50**, 760 (1994).

31. This work was funded by the Medical Research Council. We thank J. E. Walker for helpful discussions of the manuscript, the European Synchrotron Radiation Facility for provision of synchrotron radiation facilities, and W. Shepard for assistance in using beamline ID29.

Supporting Online Material

www.sciencemag.org/cgi/content/full/309/5735/771/DC1
Materials and Methods
Fig. S1
Table S1
References

25 April 2005; accepted 16 June 2005
10.1126/science.1113988

Recognition of Host Immune Activation by *Pseudomonas aeruginosa*

Licheng Wu,[1] Oscar Estrada,[1] Olga Zaborina,[1] Manjeet Bains,[4] Le Shen,[2] Jonathan E. Kohler,[1] Nachiket Patel,[1] Mark W. Musch,[3] Eugene B. Chang,[3] Yang-Xin Fu,[2] Michael A. Jacobs,[5] Michael I. Nishimura,[1] Robert E. W. Hancock,[4] Jerrold R. Turner,[2] John C. Alverdy[1]*

It is generally reasoned that lethal infections caused by opportunistic pathogens develop permissively by invading a host that is both physiologically stressed and immunologically compromised. However, an alternative hypothesis might be that opportunistic pathogens actively sense alterations in host immune function and respond by enhancing their virulence phenotype. We demonstrate that interferon-γ binds to an outer membrane protein in *Pseudomonas aeruginosa*, OprF, resulting in the expression of a quorum-sensing dependent virulence determinant, the PA-I lectin. These observations provide details of the mechanisms by which prokaryotic organisms are directly signaled by immune activation in their eukaryotic host.

Although opportunistic infection has been traditionally viewed as a passive phenomenon in which exploitative pathogens invade a weakened host, recent advances in the understanding of bacterial virulence gene regulation would suggest that this process is much more complex than previously appreciated (*1*). Because bacteria are constantly assessing the cost versus benefit of expressing virulence (*2*), it is logical that they might develop a contingency-based system to recognize physiological and immunological disturbances in their hosts. Although host cells are known to express receptors that bind bacteria for the purpose of activating the immune system, it must be considered that bacteria themselves might possess specialized receptors that in turn recognize and respond to host immune activation.

We studied this possibility using the human opportunistic pathogen, *Pseudomonas aeruginosa*, because its virulence gene regulation is well studied. We used the type I *P. aeruginosa* lectin (PA-I or *lecA*), an adhesin of *P. aeruginosa*, as a representative readout for virulence expression in this organism. Previously, we demonstrated that within the intestinal tract of a stressed host, the lethality of *P. aeruginosa* is dependent on the expression of the PA-I lectin, which causes increased permeability to its lethal cytotoxins across the intestinal epithelium (*3*). PA-I has also been shown to induce apoptosis in respiratory epithelial cells, which suggests that PA-I may be directly cytotoxic to epithelial cells (*4*). Finally, the expression of PA-I (*lecA*) is dependent on the quorum-sensing (QS) signaling system (*5*), a core system of virulence gene regulation that controls multiple virulence genes in *P. aeruginosa*.

We considered that immune elements might directly activate the virulence of *P. aeruginosa.* As a physiologically relevant in vitro source of such immune factors, supernatants from antigen-stimulated T cells, which express an array of cytokines (*6*), were evaluated for their ability to increase PA-I expression in *P. aeruginosa* strain PLL-EGFP/27853, a PA-I-GFP reporter (*7*) that was readily available and verified in a previous report by our laboratory (*8*). PA-I expression was increased by supernatant from activated T cell cultures, as assessed by enhancement of fluorescence in the PA-I-GFP fusion reporter strain (Fig. 1A), but not in controls. To determine whether this effect was due to specific cytokines, the reporter strain was individually exposed to human IL-2, IL-4, IL-6, IL-8, IL-10, IL-12, interferon gamma (IFN-γ), and tumor necrosis factor alpha (TNF-α). Of these, only IFN-γ induced a significant increase in PA-I expression that started at early stationary phase of growth (Fig. 1B). None of the cytokines tested had any significant effect on bacteria growth (Fig. 1C). Immunodepletion of IFN-γ resulted in the complete loss of its PA-I inducing capacity (Fig. 1A), which suggests that IFN-γ was the critical component in the activated T cell media that induced PA-I expression (Fig. 1A). Next, we examined PA-I expression in the completely genomically sequenced strain of *P. aeruginosa*, PAO1 (*9*), after exposure to human recombinant IFN-γ, TNF-α, IL-2, IL-4, IL-8, and IL-10 (*7*). Northern blot analysis revealed that only IFN-γ was capable of inducing *lecA* gene transcription (Fig. 1D).

Virulence in *P. aeruginosa* is highly regulated by the QS signaling system, a hierarchical system of virulence gene regulation that is dependent on bacterial cell density and growth phase (*10*–*12*). To determine the effect of growth phase on the response of *P. aeruginosa*

Departments of [1]Surgery, [2]Pathology, [3]Medicine, University of Chicago, Pritzker School of Medicine, Chicago, IL 60637, USA. [4]Department of Microbiology and Immunology, University of British Columbia, Vancouver, BC V6T1Z4, Canada. [5]Department of Genome Sciences and Medicine, University of Washington, Seattle, WA 98195, USA.

*To whom correspondence should be addressed. E-mail: jalverdy@surgery.bsd.uchicago.edu

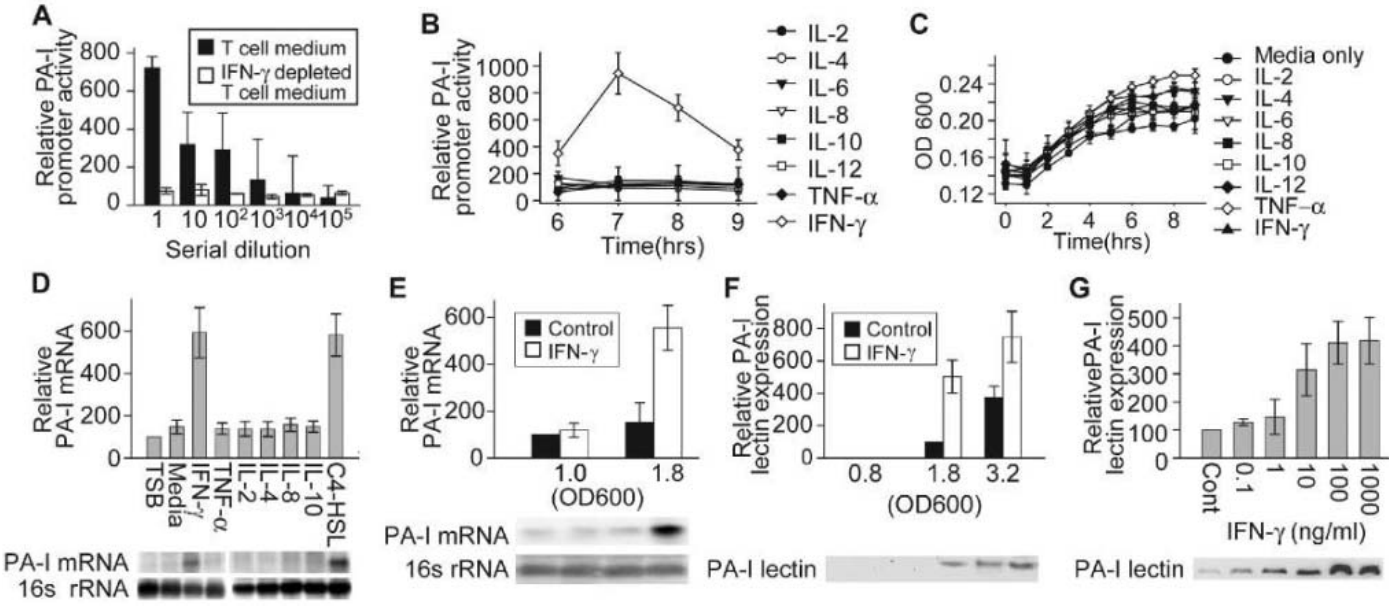


Fig. 1. IFN-γ induces the expression of the PA-I lectin in *P. aeruginosa*. Error bars, mean ± SD. (**A**) PLL-EGFP/27853 was exposed to media from activated T cells, and PA-I expression was assessed. Immunodepletion of the media with antibody to IFN-γ abolished the PA-I inducing effect by activated T cell culture media (maximum at 7 hours). (**B**) Only IFN-γ induced PA-I promoter activity after exposure of various cytokines to the GFP-PA-I reporter strain. (**C**) Optical density of *P. aeruginosa* showed that *P. aeruginosa* reached stationary growth phase at 6 hours. (**D**) *P. aeruginosa* (PAO1) was incubated with 200 ng/ml IFN-γ, TNF-α, IL-2, IL-4, IL-8, and IL-10 in cell culture media for 4 hours, and PA-I mRNA was measured by Northern blot. Induction of PA-I mRNA was observed only in the presence of IFN-γ and C_4-HSL. (**E**) *P. aeruginosa* harvested at 2 hours (OD_{600} = 1.0) and 4 hours (OD_{600} = 1.8) in the presence of 200 ng/ml IFN-γ in cell culture media. Northern blotting demonstrated that PA-I mRNA was significantly increased at early stationary phase of growth (OD_{600} = 1.8). (**F**) PA-I expression was induced following exposure to IFN-γ during stationary phase of growth, an effect not observed during log phase of growth. (**G**) Dose-dependent enhancement of PA-I expression after exposure to IFN-γ for 6 hours.

to IFN-γ, bacteria were harvested at various growth phases after exposure to IFN-γ, and PA-I mRNA and protein were measured (*7*). Both transcription and translation of PA-I increased in response to IFN-γ, starting at early stationary phase of growth (Fig. 1E and F). PA-I protein expression was also dose dependent (Fig. 1G). Taken together, these results suggest that PA-I expression in *P. aeruginosa* is enhanced in the presence of IFN-γ in a growth-dependent manner.

To determine whether IFN-γ induced PA-I by activation of the QS signaling system, we measured *rhlI* gene expression in response to IFN-γ (*7*). *RhlI* is the gene required for the synthesis of C_4-HSL (C_4-homoserine lactone), a core QS signaling molecule that plays a central role in the expression of PA-I (*5*). IFN-γ induced *rhlI* transcription in PAO1 (Fig. 2, A and B), and C_4-HSL synthesis increased significantly after exposure to IFN-γ (Fig. 2C). Activation of the QS system by IFN-γ also led to the increase of pyocyanin (PCN), another QS-dependent virulence product (*13*) (Fig. 2D). In addition, *rhlI* and *rhlR* were required for the production of PCN and PA-I expression in response to IFN-γ, because the increase of these two virulence factors by IFN-γ was abolished in mutant strains (Fig. 2, E and F). Finally, supernatant from *P. aeruginosa* exposed to IFN-γ, but not controls, altered the barrier function of cultured epithelial cells (fig. S1). Taken together, these data suggest that the QS system plays a key role in the response of *P. aeruginosa* to IFN-γ and that IFN-γ can shift the virulence of *P. aeruginosa* against epithelial cells.

We next hypothesized that IFN-γ may directly bind to a protein on the surface of *P. aeruginosa*, leading to virulence up-regulation. Consistent with this, we observed that IFN-γ avidly bound to whole fixed cells of *P. aeruginosa* in a dose-dependent manner (Fig. 3A). The vast majority of bacterial cells (73% ± 3.2% versus 8.5% ± 2.5%) bound IFN-γ (Fig. 3B and fig. S2). The binding capacity of the IFN-γ to *P. aeruginosa* was not affected significantly by the growth phase of bacteria (fig. S3A). To determine whether IFN-γ bound to membrane or cytosolic fractions of *P. aeruginosa*, equal protein concentrations of each fraction were prepared (*7*), and results showed that IFN-γ preferentially bound to membrane fractions by enzyme-linked immunosorbent assay (ELISA) (fig. S3B). Furthermore, IFN-γ binding to *P. aeruginosa* membranes was diminished upon proteinase K treatment (fig. S3C), which suggests that IFN-γ binds to a protein on the bacterial cell membrane. Binding was specific to IFN-γ, because no binding was observed with any other cytokines tested (fig. S3D). Taken together, these data indicate that IFN-γ binds specifically to a membrane protein (s) on *P. aeruginosa*.

P. aeruginosa membrane proteins solubilized with mild detergents (*7*) retained their binding capacity to IFN-γ (Fig. 3C), thus making it possible to isolate the putative binding protein by immunoprecipitation. Membrane proteins were next separated by nondenaturing gel electrophoresis, transferred to polyvinylidene difluoride membranes, and hybridized with IFN-γ followed by biotin-labeled antibody to IFN-γ; results revealed a single immunoreactive band at 35 kD that was dependent on the dose of IFN-γ (Fig. 3D). Immunoprecipitation against the *P. aeruginosa* fractionated membrane protein isolated a 35-kD protein that was IFN-γ dependent (Fig. 3E). Use of ESI-TRAP LC-MS-MS ion trap (electrospray ionization–telomeric repeat amplification protocol liquid chromatography tandem mass spectrometry) identified the 35-kD protein to be the *P. aeruginosa* outer membrane porin OprF (Fig. 3F) (*14*). We next verified that OprF was a major binding site for IFN-γ by showing that solubilized membrane proteins from OprF mutant strains (*15*) displayed reduced binding to IFN-γ (Fig. 4A). Immunoprecipitation of solubilized membrane protein confirmed the role of OprF by showing complete loss of the ~35-kD band in the OprF mutant strain (Fig. 4B). Further evidence supporting the role of OprF in the IFN-γ response was found when mutant strains failed to increase PA-I protein expression after exposure to an effective stimulating dose of IFN-γ as compared with the wild-type strain (Fig. 4, C and D). When OprF was reconstituted in the mutant strain 31899 using the plasmid pUCP24/OprF,

responsiveness to IFN-γ was reestablished, with an increase in PA-I protein expression (Fig. 4E). Finally, ELISA binding assays between purified OprF and IFN-γ demonstrated that OprF binds directly to human IFN-γ (Fig. 4F) in a dose-dependent manner.

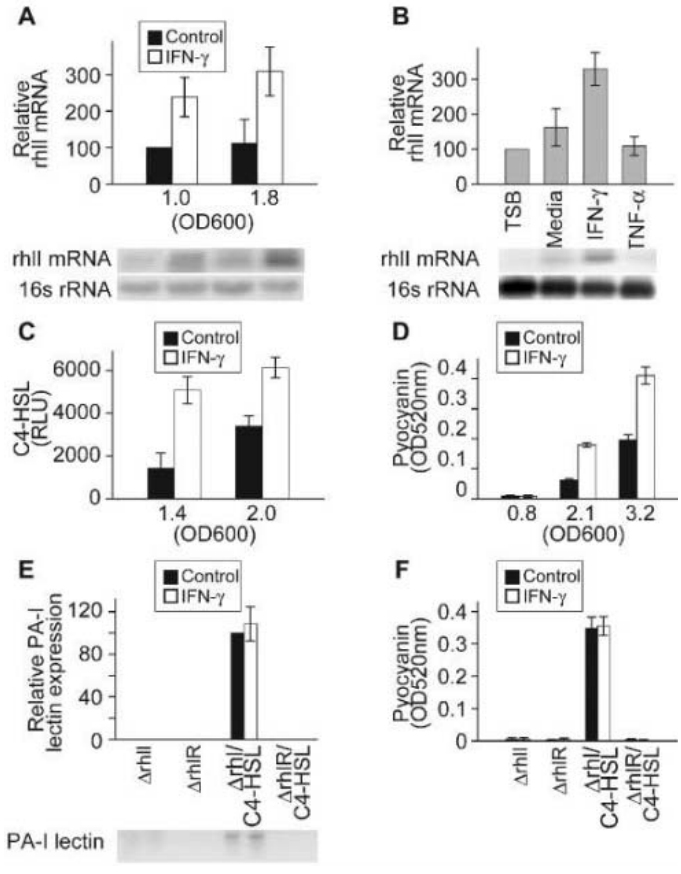


Fig. 2. The presence of *rhlI* and *rhlR*, core QS signaling elements in *P. aeruginosa*, are required for PA-I expression and pyocyanin production in response to IFN-γ. Error bars, mean ± SD. (**A**) *P. aeruginosa* harvested at 2 hours (OD_{600} = 1.0) and 4 hours (OD_{600} = 1.8) after incubation with 200 ng/ml IFN-γ in cell culture media. Northern blotting demonstrated that IFN-γ increased *rhlI* mRNA levels significantly. (**B**) IFN-γ, but not TNF-α, induced the transcription of *rhlI* mRNA. (**C**) The gene product of *rhlI*, C_4-HSL, a key diffusible QS signaling molecule, was measured by the luminescence reporter strain pSB536 in PAO1 supernatant and was increased following exposure to IFN-γ. (**D**) Pyocyanin, an additional QS-dependent virulence factor, was also up-regulated in PAO1 in the presence of 100 ng/ml of IFN-γ. (**E**) Immunoblots of PA-I expression demonstrated that exposure of *rhlI* and *rhlR* mutants to IFN-γ failed to induce PA-I expression. The addition of exogenous C_4-HSL did not restore responsiveness to IFN-γ in either mutant. (**F**) Pyocyanin production by IFN-γ required the presence of *rhlI* and *rhlR*, because mutants did not produce pyocyanin when exposed to IFN-γ. Adding C_4-HSL to the *rhlI* and *rhlR* mutants did not restore responsiveness to IFN-γ.

Although it was previously recognized that various cytokines, including IL-1β (*16*) and TNF-α (*17*), can affect the growth and virulence properties of bacteria, little progress had been made on the mechanistic details of these initial observations. For example, although TNF-α has been shown to display high-affinity binding to *Shigella flexneri*, the specific receptor that mediates this response has not yet been identified. In addition, although TNF-α has been shown to induce *Shigella flexneri* to become more invasive against cultured epithelial cells, the specific signaling pathways and virulence factors that mediate this response remain unknown. The observation here that *P. aeruginosa* binds INF-γ through OprF, resulting in activation of the QS system, provides specific information on the mechanisms by which certain bacteria sense and respond to the host immune system. That IFN-γ binding to OprF induces *P. aeruginosa* to express both PA-I and pyocyanin, both of which are capable of disrupting epithelial cell function, is intriguing given that, in contradistinction to TNF-α, the main function of IFN-γ is bacterial clearance (*18*). Data from the present study provide molecular evidence that certain opportunistic pathogens such as *P. aeruginosa* may have evolved a contingency-based mechanism to mount an effective countermeasure to immune activation by their host. It remains to be clarified whether other virulence regulators that activate the QS system, such as the quinolone signaling system (*19*, *20*) and the lasRI system (*5*), may be involved in the transduction of membrane signaling in *P. aeruginosa* to IFN-γ. Finally, the design of appropriate animal models using IFN-γ knockout mice and OprF mutant strains will be

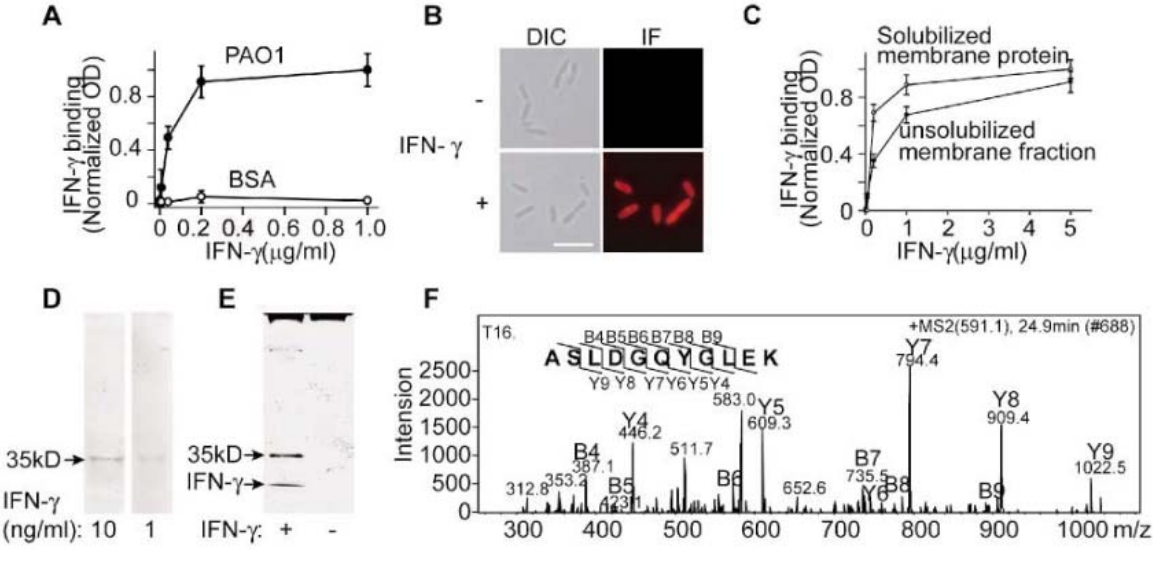


Fig. 3. Identification of the IFN-γ binding site from solubilized membrane fractions of *P. aeruginosa* (PAO1). Error bars, mean ± SD. (**A**) ELISA assay demonstrated a dose-dependent binding of IFN-γ to *P. aeruginosa*. (**B**) Epifluorescence photomicrographs of IFN-γ bound to whole cells of *P. aeruginosa*. Binding was detected using biotin-labeled antibody to IFN-γ and fluorescence Alexa 594–labeled streptavidin. DIC, digital intense phase contrast; IF, immunofluorescence; scale bar, 5 μm. (**C**) ELISA assay demonstrated that IFN-γ binds to solubilized membrane proteins from *P. aeruginosa*. (**D**) Solubilized membrane proteins separated by nondenatured polyacrylamide gel electrophoresis were detected using IFN-γ as the first antibody. Representative immunoblot (n = 3) demonstrated dose-dependent IFN-γ binding to a single 35-kD solubilized membrane protein. (**E**) Immunoprecipitation of the solubilized membrane protein with IFN-γ and antibody to IFN-γ resulted in a distinctive band. (**F**) MS-MS spectra identified the 35-kD protein as OprF (outer membrane protein F).

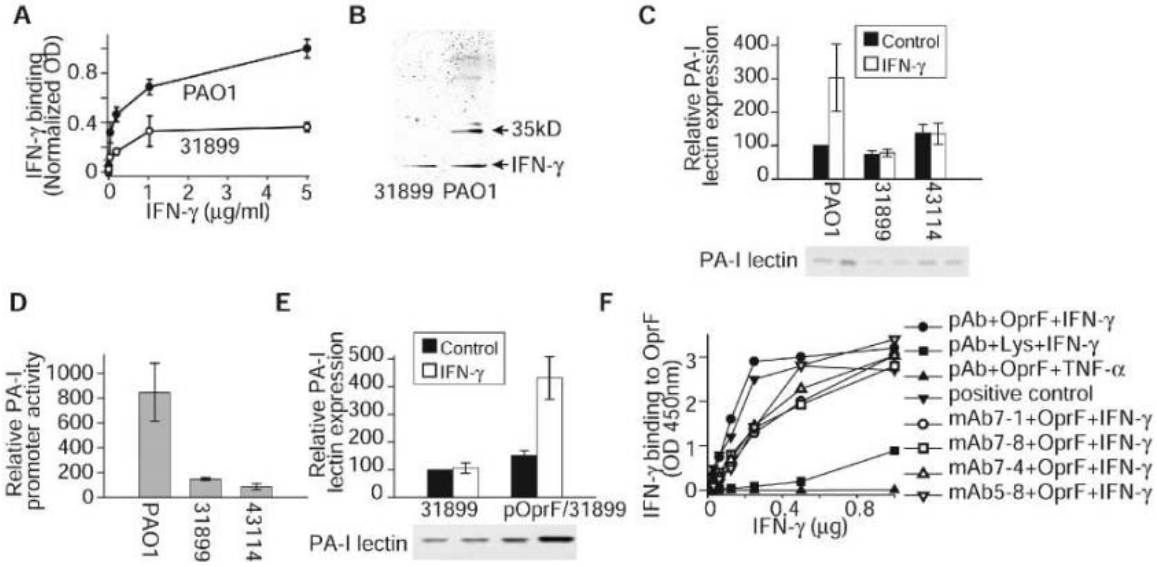


Fig. 4. IFN-γ binds to OprF and induces PA-I expression. Error bars, mean ± SD. (**A**) ELISA binding assays of IFN-γ to solubilized membrane protein from wild-type *P. aeruginosa* (PAO1) and the OprF knockout mutant strain 31899 showing attenuated IFN-γ binding to the solubilized membrane protein from the mutant strain. (**B**) Immunoprecipitation of solubilized membrane proteins from OprF mutant strain 31899 with IFN-γ, demonstrating absence of the 35-kD band seen with the parent wild-type strain (PAO1). (**C**) PA-I protein expression measured by immunoblot in wild-type (PAO1) and mutant strains (31899, 43114) exposed to 200 ng/ml IFN-γ, demonstrating an inability of IFN-γ to enhance the expression of PA-I in the OprF mutant strains. (**D**) Wild-type strain (PAO1) and OprF mutant strains (31899, 43114) carrying the GFP-PA-I fusion plasmid were incubated with 200 ng/ml IFN-γ, and fluorescence was assessed over time. Results demonstrate a lack of enhanced PA-I expression in mutants exposed to IFN-γ. (**E**) Reconstitution of OprF in mutant strain 31899 demonstrating reestablishment of the responsiveness of PA-I expression to IFN-γ. (**F**) Antibody to OprF (polyclonal, pAb; monoclonal, mAb) was coated onto microtiter plate. The complexes [OprF and IFN-γ, IFN-γ and Lys (lysozyme), and OprF and TNF-α] were added and detected by biotin-labeled antibody to IFN-γ. ELISA assay demonstrated that human IFN-γ binds to purified OprF. Results are a representative experiment of three independent studies.

necessary to confirm the role of IFN-γ binding to OprF on *P. aeruginosa* virulence in vivo.

References and Notes

1. H. Ochman, N. A. Moran, *Science* **292**, 1096 (2001).
2. J. J. Mekalanos, *J. Bacteriol.* **174**, 1 (1992).
3. R. S. Laughlin *et al.*, *Ann. Surg.* **232**, 133 (2000).
4. O. Bajolet-Laudinat *et al.*, *Infect. Immun.* **62**, 4481 (1994).
5. K. Winzer *et al.*, *J. Bacteriol.* **182**, 6401 (2000).
6. D. J. Schwartzentruber, S. L. Topalian, M. Mancini, S. A. Rosenberg, *J. Immunol.* **146**, 3674 (1991).
7. Materials and methods are available as supporting material on *Science* Online.
8. L. Wu *et al.*, *Gastroenterology* **126**, 488 (2004).
9. C. K. Stover *et al.*, *Nature* **406**, 959 (2000).
10. S. P. Diggle, K. Winzer, A. Lazdunski, P. Williams, M. Camara, *J. Bacteriol.* **184**, 2576 (2002).
11. M. Whiteley, K. M. Lee, E. P. Greenberg, *Proc. Natl. Acad. Sci. U.S.A.* **96**, 13904 (1999).
12. V. E. Wagner, D. Bushnell, L. Passador, A. I. Brooks, B. H. Iglewski, *J. Bacteriol.* **185**, 2080 (2003).
13. G. W. Lau, H. Ran, F. Kong, D. J. Hassett, D. Mavrodi, *Infect. Immun.* **72**, 4275 (2004).
14. A. O. Azghani, S. Idell, M. Bains, R. E. Hancock, *Microb. Pathog.* **33**, 109 (2002).
15. M. A. Jacobs *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **100**, 14339 (2003).
16. R. Porat, B. D. Clark, S. M. Wolff, C. A. Dinarello, *Science* **254**, 430 (1991).
17. G. Luo, D. W. Niesel, R. A. Shaban, E. A. Grimm, G. R. Klimpel, *Infect. Immun.* **61**, 830 (1993).
18. S. Ikuta *et al.*, *Shock* **21**, 452 (2004).
19. S. P. Diggle *et al.*, *Mol. Microbiol.* **50**, 29 (2003).
20. E. C. Pesci *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **96**, 11229 (1999).

21. We thank D. R. Clayburgh for assistance with the figure preparations, P. Williams and K. Winzer for providing *Escherichia coli* strain harboring the reporter plasmid pSB536, and H. P. Schweizer for providing plasmid pUCP24. J.C.A. and E.B.C. are founding members and shareholders of Midway Pharmaceuticals, which is involved in developing compounds that interfere with host compounds released during stress and bacterial receptors. This work was supported by NIH grants 2-RO1 GM062344-05 (J.C.A.); R01DK61931 and R01DK68271 (J.R.T.); DK-47722 and DK-38510 (E.B.C.); and by the Canada Research Chairs Program and the Canadian Institutes of Health Research (R.E.W.H.).

Supporting Online Material
www.sciencemag.org/cgi/content/full/309/5735/774/DC1

Materials and Methods
Figs. S1 to S3
References

17 March 2005; accepted 2 June 2005
10.1126/science.1112422

A Phenylalanine Clamp Catalyzes Protein Translocation Through the Anthrax Toxin Pore

Bryan A. Krantz,[1]* Roman A. Melnyk,[1]* Sen Zhang,[1] Stephen J. Juris,[1] D. Borden Lacy,[1] Zhengyan Wu,[2] Alan Finkelstein,[2] R. John Collier[1]†

The protective antigen component of anthrax toxin forms a homoheptameric pore in the endosomal membrane, creating a narrow passageway for the enzymatic components of the toxin to enter the cytosol. We found that, during conversion of the heptameric precursor to the pore, the seven phenylalanine-427 residues converged within the lumen, generating a radially symmetric heptad of solvent-exposed aromatic rings. This "ϕ-clamp" structure was required for protein translocation and comprised the major conductance-blocking site for hydrophobic drugs and model cations. We conclude that the ϕ clamp serves a chaperone-like function, interacting with hydrophobic sequences presented by the protein substrate as it unfolds during translocation.

Anthrax toxin is composed of three nontoxic proteins, which combine on eukaryotic cell surfaces to form toxic, noncovalent complexes. [See (*1*) for a review.] Protective antigen (PA), the protein translocase component, binds to a cellular receptor and is activated by a furin-family protease. The resulting 63-kD receptor-bound fragment, PA_{63}, self-assembles into the prepore, which is a ring-shaped homoheptamer (Fig. 1A). The prepore then forms complexes with the two ~90-kD enzymatic components, lethal factor (LF) and edema factor (EF). These complexes are endocytosed and delivered to an acidic compartment (*2*). There, the prepore undergoes an acidic pH-dependent conformational rearrangement (*3*) to form an ion-

conducting, cation-selective, transmembrane pore (*4*), allowing bound LF and EF to translocate into the cytosol.

The PA_{63} pore (Fig. 1B) is believed to consist of a mushroom-shaped structure, with a globular cap connected to a β-barrel stem that is ~100 Å long (*5*, *6*). A model of the 14-strand β barrel reveals its lumen, which is ~15 Å wide and can only accommodate structure as wide as an α helix (*7*). The narrow pore creates a structural bottleneck, requiring that the catalytic factors, LF and EF, unfold in order to be translocated (*8*, *9*). The destabilization energy required to unfold the tertiary structure of LF and EF originates partly from the acidic pH in endosomes, which causes their N-terminal domains (LF_N and EF_N) to become molten globules (MG) (*7*). A positive membrane potential [$+\Delta\psi$ (*10*)], when coupled with these acidic pH conditions, is sufficient to drive LF_N through PA_{63} pores formed in planar lipid bilayers (*9*). To enter the narrow confines of the ~15-Å-wide lumen, LF_N must shed its residual tertiary structure and convert from the MG form to an extended, "translocatable" conformation (*7*). How does a solvent-filled pore mediate the disassembly of an MG protein, packed, albeit loosely, with hydrophobically dense stretches of polypeptide? We surmised that an interaction surface inside the pore might facilitate further unfolding of the MG to the extended, translocatable form.

By cysteine-scanning mutagenesis coupled to [2-(trimethylammonium) ethyl]methanethiosulfonate (MTS-ET) modification (*5*) (Fig. 1C), we identified residues that line the lumen of PA_{63} in the globular cap portion of domain 2, the pore-forming domain (Fig. 1D). F^{427} (*11*) was the most hydrophobic residue identified in the otherwise hydrophilic pore lining. It is absolutely conserved in homologous toxins (fig. S1), and mutating it blocks protein translocation (*12*, *13*). $F^{427}\rightarrow C^{427}$ (F427C) channels were most strongly affected by MTS-ET modification, implying that F^{427} is prominent and solvent-exposed within the lumen.

To address how the seven F^{427} residues were arranged within the lumen of the PA_{63} pore, we used electron paramagnetic resonance spectroscopy (EPR) to measure the proximity of nitroxide spin labels attached to F427C. The EPR spectrum of the spin-labeled prepore showed weak spin-spin interactions (Fig. 1E). This observation was consistent with the crystal structure of the prepore, in which F^{427} lies in a disordered loop (2β10 to 2β11) near the lumen, such that neighboring F^{427} residues are 15 to 20 Å apart (Fig. 1A). Upon conversion to the pore state by acidification to pH 6, a saturating spin-spin interaction appeared, indicating that the spin probes had converged and were separated by less than 10 Å (Fig. 1E). Consistent with this, single-channel ion conductances were roughly inversely proportional to the size of the substitution at 427 (Fig. 1F), as predicted from a cylindrical pore conductance model (*14*). Channels with large aliphatic (Leu) or aromatic (Trp) residues at 427 showed smaller conductances than channels with Ala or Phe, their smaller respective counterparts. Thus, as the pore forms, the seven phenylalanines create a narrow aromatic iris, or "ring of rings," within the lumen (Fig. 1B and supporting online material text).

In planar lipid bilayers, LF_N binds to wild-type (WT) PA_{63} pores, blocking ion conductance by >95% (at +20 mV and ~10 nM LF_N; Fig. 2C); the first 21 residues of LF_N's cation-rich, flexible N terminus are essential for this macroscopic blocking effect (*15*). At the single-channel level, the N terminus of LF_N bound stably within the PA_{63} channel and prevented passage of hydrated K^+ ions, as manifested by a continuously closed state (Fig. 2A). When F427A PA_{63} was similarly assayed, LF_N was only able to block macroscopic conductance by ~50% (Fig. 2C). In single-channel recordings, the partial block observed for F427A PA_{63} channels resulted from a dynamic "flickering" between an open state, multiple partly closed substates, and a fully closed state (Fig. 2A). Histogram analysis of a single-channel recording of F427A PA_{63} (Fig. 2B) was consistent with macroscopic experiments, in that the average conductance was ~50% of the open channel conductance (Fig. 2C). We infer that LF_N is bound to the domain 1′ surface of the F427A channel, but its flexible N terminus, which enters the channel first (*15*), is not stably bound within the lumen and is unable to block conductance effectively. LF_N blocked PA_{63} pores more effectively with large aromatic or aliphatic residues at position 427 than with small or hydrophilic residues (fig. S2). Thus the heptad of F^{427} residues is integral to binding LF_N's N terminus, leading us to term the site the "ϕ clamp."

To probe the role of the ϕ clamp in polypeptide translocation, we measured the rate of LF_N translocation through PA_{63} channels formed in planar lipid bilayers (*9*). LF_N was added to the cis compartment, blocking conductance. After perfusing to remove unbound LF_N, we stepped $\Delta\psi$ to a higher positive voltage and monitored the rate of translocation by the increase in channel conductance as LF_N

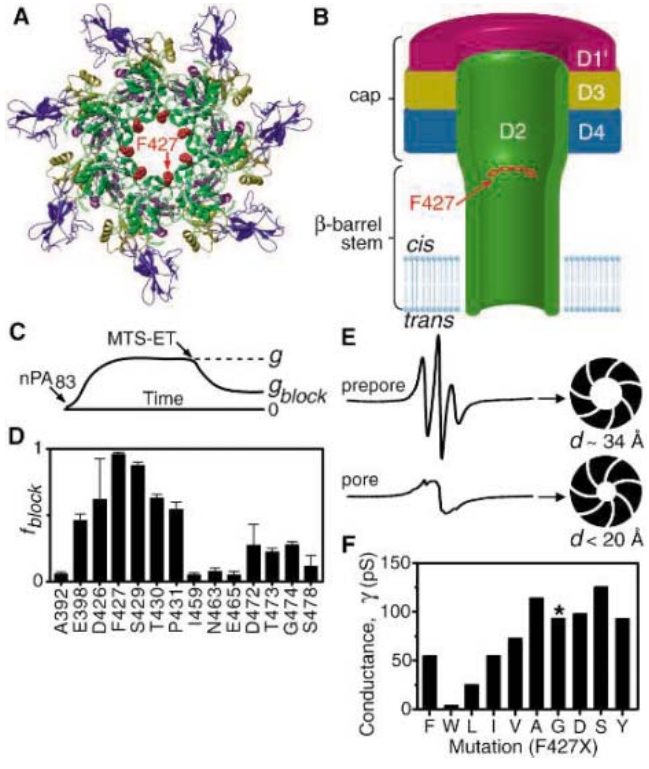


Fig. 1. Structural models of a lumen-facing phenylalanine heptad. (**A**) A ribbons rendering of the PA_{63} prepore (*27*), viewed axially, where domain 4 is proximal. Domains are colored: D1′ (magenta), D2 (green), D3 (gold), and D4 (blue). F427 (red, space filling) is modeled into the structure. (**B**) Hypothetical cross section of the PA_{63} channel, or pore, colored as in (A). The membrane-spanning tube is the 14-stranded β barrel from domain 2 (*5*, *6*). (**C**) Illustration of the effect of MTS-ET modification on Cys-substituted mutants of PA_{63} in macroscopic conductance studies. Conductance, *g*, is determined from the current, *I*, and $\Delta\psi$ as $g = I/\Delta\psi$. (**D**) Fraction of conductance blocked (f_{block}) by MTS-ET modification (*28*) in domain 2 cap residues [as in (C), where $f_{block} = 1 - g_{block}/g$] (table S2). Error bars show means + σ_{SE} ($n = 3$). (**E**) EPR spectra of PA_{63} heptamers uniformly labeled at F427C with a Cys-reactive nitroxide spin label in the prepore state at pH 8.5 (upper spectrum) and the pore state at pH 6 (lower spectrum). Approximate luminal diameters, *d*, are based on the observed spin-spin interactions. (**F**) Unitary conductance, γ, of single PA_{63} channels, with indicated substitutions at F^{427}. Channels formed by F427G PA_{63} (*) initially opened to a conductance of 90 pS, but, unlike any of the other channels, flickered to 60 and 30 pS substates. γ values are accurate to at least ±10%, except for F427L and F427W, which are accurate to ±20%.

[1]Department of Microbiology and Molecular Genetics, Harvard Medical School, 200 Longwood Avenue, Boston, MA 02115, USA. [2]Department of Physiology and Biophysics, Albert Einstein College of Medicine, 1300 Morris Park Avenue, Bronx, NY 10461, USA.

*These authors contributed equally to this work.
†To whom correspondence should be addressed. Email: jcollier@hms.harvard.edu

traversed the pore (Fig. 2D). With the most active substitutions at F^{427}, LF_N translocated at rates that trended as Phe > Leu ≈ Trp > Tyr (Fig. 2D). Other small aliphatic or hydrophilic substitutions, including Asp (an isosteric, hydrophilic control for Leu), were inefficient at promoting translocation. The most active residue, therefore, was Phe; the more hydrophilic aromatic, Tyr, was five times less active. These in vitro translocation studies recapitulated results of cellular assays of toxin action (Fig. 2E). Preference at the ϕ-clamp site for the γ-branched Leu over its β-branched isomer, Ile, correlates with the fact that aromatic residues also place more hydrophobic surface nearer to the center of the lumen. Thus the ϕ-clamp structure represents an active site, requiring either an aromatic surface or a more centrally oriented aliphatic surface to catalyze translocation efficiently.

Considering the hydrophobicity of the ϕ clamp, we hypothesized that this site may also be the binding site for hydrophobic cations, such as tetrabutylammonium (TBA) (Fig. 3A) (*16*). This hypothesis is consistent with studies suggesting that the TBA site is within the cap of the PA_{63} channel, cis to the extended β barrel (*6*). TBA's affinity for F427A channels was greatly reduced from that of WT (4000-fold, or ~5 kcal mol^{-1}; Fig. 3D). Large aliphatic residues at 427 were unable to recover the TBA block (Fig. 3E). F427L channels, for example, had a ~4 kcal mol^{-1} reduction in affinity for TBA. The least defective substitution was F427Y, which reduced the TBA block fourfold, or ~0.8 kcal mol^{-1}. The recovery of the TBA block in F427Y channels was notable, because Tyr is substantially more hydrophilic than Phe. We infer that the TBA blocking mechanism also includes cation-π

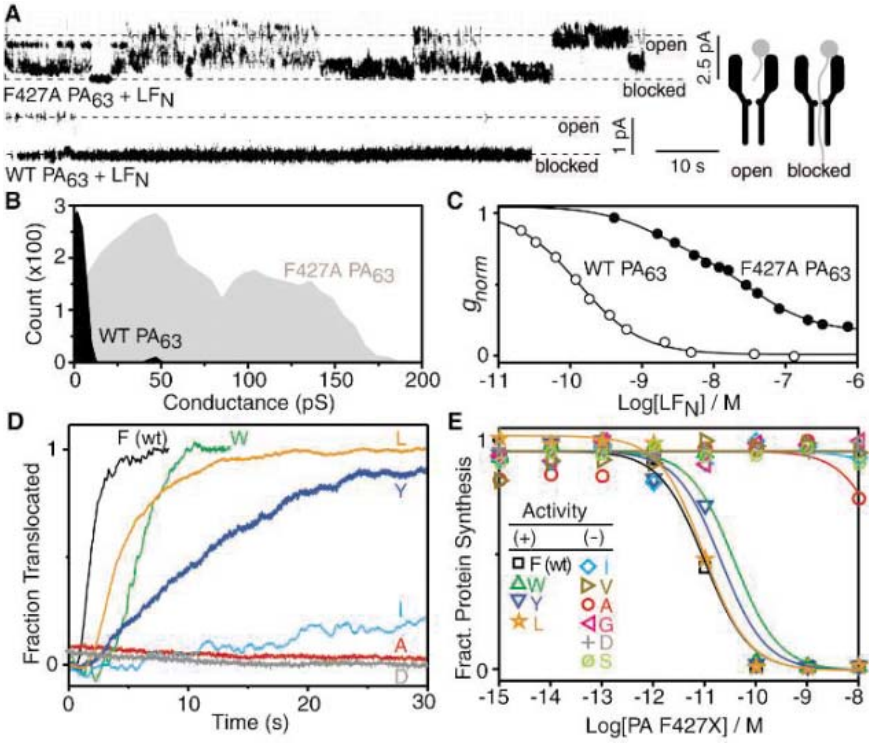


Fig. 2. LF_N conductance block and translocation studies with PA_{63} mutated at F^{427}. (**A**) Current record for a single F427A PA_{63} channel (upper panel) exhibiting partial block with 7 nM LF_N in the cis compartment. A similar single-channel record for a WT channel (lower panel) shows complete block with 7 nM LF_N in the cis compartment [$\Delta\psi$ = +20 mV (*10*), pH 5.5]. A diagram (right) showing the "blocked" channel corresponds to LF_N's N terminus binding within the pore. (**B**) Histogram of the single-channel conductances for the LF_N block in (A) for WT (black) and F427A PA_{63} (gray). (**C**) Concentration dependence of the LF_N conductance block for WT (○) and F427A PA_{63} channels (●) under macroscopic conditions ($\Delta\psi$ = +20 mV, pH 5.5). Normalized conductance (g_{norm}) curves are fitted to a single- and two-site binding model for WT and F427A channels, respectively, where the WT equilibrium dissociation constant (K_D) is ~150 pM. (**D**) Planar lipid bilayer macroscopic conductance records of LF_N translocation through WT and the indicated F^{427} mutant channels [where pH_{cis} = 5.5, pH_{trans} = 6.5 (*29*)]. After PA_{63}-induced channel formation reached steady state, 20 nM LF_N was added to the cis compartment (under a $\Delta\psi$ of +1 to +10 mV). When the conductance block reached steady state, the cis compartment was perfused, and translocation (as manifested by the rise in conductance) was initiated by increasing $\Delta\psi$ to +30 mV. Plotted records were normalized as the fraction translocated, excepting F427A and F427D channels, in which no appreciable translocation occurred. (**E**) Cellular translocation assay of F427 PA mutants (*12*), where translocation is reported by detecting the inhibition of protein synthesis by the domain from diphtheria toxin, which is fused to the C terminus of LF_N. Mutants are classified as active (+) or inactive (–).

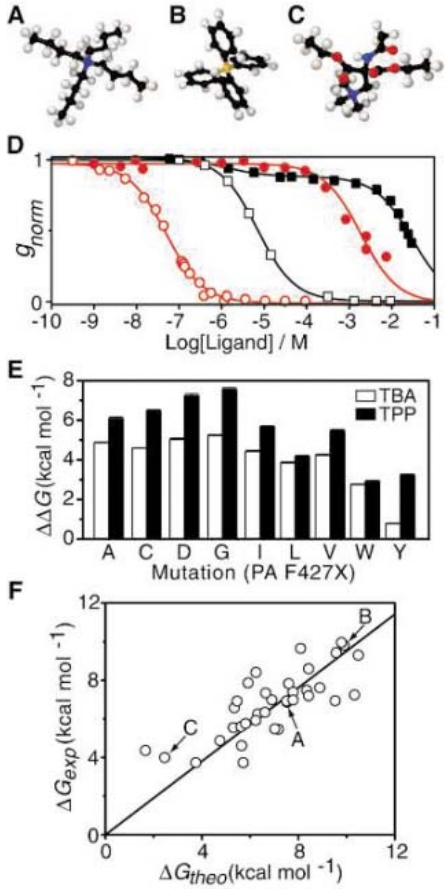


Fig. 3. QAP conductance block at the ϕ-clamp site. (**A**) TBA, (**B**) TPP, and (**C**) a hydrophilic analog of TBA (2-acetylamino-2,2-bis-ethoxy-carbonyl-ethyl)-trimethyl-ammonium, colored by atom: C (black), N (blue), O (red), H (white), and P (orange). (**D**) QAP ions were added symmetrically to the cis and trans compartments ($\Delta\psi$ = +20 mV, pH 5.5), and g_{norm} was recorded once QAP binding reached equilibrium. For TPP block, single-site binding models were fit for WT PA_{63} (red ○), K_D = 46 ± 2 nM (±SD), and F427A PA_{63} channels (red ●), K_D = 1.7 ± 0.2 mM. For TBA block, a single-site binding model was fit for WT PA_{63} (black □), K_D = 7.3 ± 0.3 μM, but a two-site binding model was required for F427A PA_{63} (black ■), such that the K_D values of the major amplitude (88%) and minor amplitude (12%) were 30 ± 2 mM and 1.5 ± 0.7 μM, respectively. (**E**) Binding free energy change, $\Delta\Delta G = RT \ln K_D^{Mut}/K_D^{WT}$, of TBA (white bars) and TPP (black bars) block for F^{427} mutants. (**F**) Model compound blocking studies of WT PA_{63} channels, performed as in (D). Experimental binding energies ($\Delta G_{exp} = RT \ln K_D$) were correlated to the expected binding energies (ΔG_{theo}) composed of solvation (ΔG_s) (*18*) and aromatic enhancement (ΔG_{aro}) energies as well as an offset, *c*, where $\Delta G_{theo} = \Delta G_s + \Delta G_{aro} + c$. $\Delta G_{aro} = \alpha n$, where α was fit to 0.7 ± 0.3 kcal mol^{-1} per aromatic ring, using the number of aromatic rings per compound, *n*. *c* was 0.8 ± 0.4 kcal mol^{-1} (table S1). The linear fit, $\Delta G_{exp} = \Delta G_{theo} \times m$ (35 compounds), had a slope, *m*, of 0.95 ± 0.03 and total error, σ_{SD}, of 1.2 kcal mol^{-1}. Arrows indicate compounds in (A), (B), and (C).

interactions, which occur when aromatic residues interact with cations through their delocalized, negative π-electron clouds (*17*).

We next examined a library of 35 quaternary ammonium and phosphonium ion (QAP) compounds to establish the nature of the ϕ-clamp binding interaction. Aliphatic QAP compounds (table S1) blocked conductance according to a solvent-accessible surface area solvation energy model (*18*); i.e., compounds with more hydrophobic surface blocked more effectively (Fig. 3F). Thus, a hydrophilic analog of TBA that is comparable in size, but functionalized with hydrophilic amide and ester groups (Fig. 3C), blocked 140-fold more weakly than TBA (table S1). We found that WT channels preferred tetraphenylphosphonium (TPP) (Fig. 3B) to TBA by 160-fold (Fig. 3D). Broadly across the QAP library, the ϕ clamp preferred aromatic moieties by ~0.7 kcal mol^{-1} per aromatic ring (Fig. 3F and table S1). Specifically, the conductance block observed for the polyaromatic, 4-aminoquinolone drug, quinacrine (*19*), was reduced ~1000-fold in F427A channels (fig. S3), indicating the ϕ-clamp site may be exploited in the development of channel-blocking drugs. Thus the correlation observed for the diverse QAP compound library (Fig. 3F and table S1) indicates that the ϕ clamp does not, in and of itself, recognize specific geometric or steric features of the substrates. Instead, the WT ϕ clamp recognizes substrates primarily by nonspecific hydrophobic interactions (*20*), although its negative π-clouds also contribute electrostatically through aromatic-aromatic, π-π and cation-π interactions.

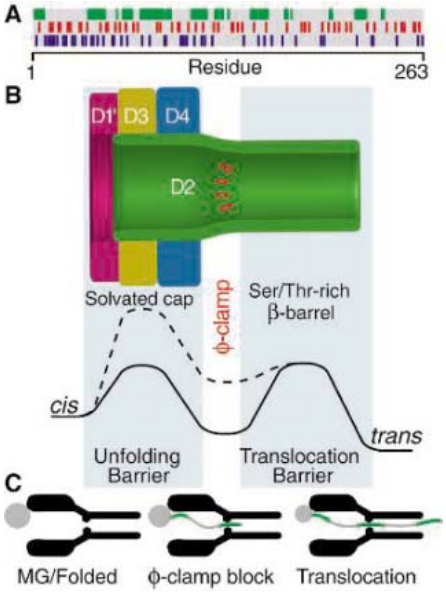


Fig. 4. A model of ϕ-clamp catalyzed protein translocation. (**A**) Chemical complexity of a PA_{63} substrate, LF_N, in which residues are colored by functionality: hydrophobic (green), greater than –1.75 kcal mol^{-1} in solvation energy (*18*) after applying a 10-residue running-window average; cationic (blue); and anionic (red). (**B**) The PA_{63} pore with a luminal ϕ-clamp site (red) is structurally imposed on an energy-well diagram for a hydrophobic stretch of polypeptide sequence from LF_N [see (A)]. The ϕ-clamp site is the anticipated well, separating the unfolding barrier on the cis side in the solvent-filled cap from a translocation barrier on the trans side in the solvophilic, Ser/Thr-rich β barrel. Energy diagrams are for WT and F427A PA_{63} (solid and broken lines, respectively), where the mutation simultaneously reduces the hydrophobic-mediated stabilization imparted by the ϕ-clamp site and raises the barrier to unfolding hydrophobic sequences from the protein substrate. (**C**) Intermediate states for a stepwise, Brownian ratchet unfolding and translocation mechanism, such that hydrophobically dense polypeptide segments (green) interact with the ϕ-clamp constriction.

In determining a model for translocation, we disfavored a priori relationships used to describe metal ion-conducting channels, because metal ion throughput (Fig. 1F) did not correlate with protein transport (Fig. 2, D and E), and the steric constraints imposed by the ϕ-clamp site did not impede protein translocation. The WT ϕ-clamp site should create a hydrophobic and steric energy barrier for bulky, hydrophilic, or charged residues in the protein substrate. Paradoxically, although channels containing narrower, aromatically lined ϕ-clamp sites were less ion conducting, they translocated LF_N orders of magnitude more rapidly than channels made wider, more hydrophilic, and more ion conducting at the ϕ-clamp site.

We therefore considered a chaperone model in which the ϕ-clamp's phenyl rings directly interacted with the translocating polypeptide. This view is supported, because the ϕ clamp forms a narrow iris (Fig. 1E) that can effectively grasp the translocating polypeptide chain (Fig. 2A, diagram). Furthermore, model compound studies indicate that the ϕ clamp recognizes substrates through the hydrophobic effect, enhanced by aromatic-aromatic, π-π, and cation-π interactions (Fig. 3 and table S1). Similarly, in the potassium ion channel from *Streptomyces lividans* (KcsA), a hydrophobic cavity defines the TBA blocking site and corresponds to a docking site for a hydrophobically dense peptide sequence (*21*). In globular proteins, like LF, hydrophobically dense peptide segments occur periodically along the translocating chain (Fig. 4A). As these segments unwind from the MG protein, they would be expected to bind favorably to the ϕ clamp, causing translocation to be blocked kinetically by the unfavorable hydrophilic barrier in the Ser/Thr-rich β barrel (Fig. 4B). Thus the ϕ-clamp energy well might be expected to hinder translocation, causing polypeptide segments bound at the site to "pause." However, as the ϕ-clamp site actually catalyzes polypeptide translocation, it must reduce some other larger barrier, such as the unfolding of the substrate protein. By analogy to the KcsA channel's selectivity filter, which provides sequential rings of hydrophilic carbonyl oxygen atoms that mimic the inner hydration shell of a K^+ ion (*22*), we propose that the ϕ-clamp site creates an environment that mimics the hydrophobic core of the unfolding MG protein. This would reduce the energetic penalty of exposing hydrophobic sequences to solvent or the hydrophilic lumen of the channel. The PA_{63} pore would thereby function as a Brownian ratchet, enabling the unwound leading segment of a translocating protein to move through the channel, and the trailing part of the protein to more readily unfold (Fig. 4C).

The results presented here show that PA_{63} is not merely a passive conduit through which proteins electrophorese; rather, it actively engages the translocating substrate via the ϕ clamp. This paradigm may be relevant to the functions of other polymer-translocating channels containing exposed hydrophobic sites, such as the "hydrophobic gasket" and "aromatic slide," identified in the structures of the protein secretase (*23*) and maltoporin (*24*) channels, respectively.

References and Notes

1. R. J. Collier, J. A. Young, *Annu. Rev. Cell Dev. Biol.* **19**, 45 (2003).
2. A. M. Friedlander, *J. Biol. Chem.* **261**, 7123 (1986).
3. C. J. Miller, J. L. Elliott, R. J. Collier, *Biochemistry* **38**, 10432 (1999).
4. R. O. Blaustein, T. M. Koehler, R. J. Collier, A. Finkelstein, *Proc. Natl. Acad. Sci. U.S.A.* **86**, 2209 (1989).
5. E. L. Benson, P. D. Huynh, A. Finkelstein, R. J. Collier, *Biochemistry* **37**, 3941 (1998).
6. S. Nassi, R. J. Collier, A. Finkelstein, *Biochemistry* **41**, 1445 (2002).
7. B. A. Krantz, A. D. Trivedi, K. Cunningham, K. A. Christensen, R. J. Collier, *J. Mol. Biol.* **344**, 739 (2004).
8. J. Wesche, J. L. Elliott, P. O. Falnes, S. Olsnes, R. J. Collier, *Biochemistry* **37**, 15737 (1998).
9. S. Zhang, E. Udho, Z. Wu, R. J. Collier, A. Finkelstein, *Biophys. J.* **87**, 3842 (2004).
10. We define the membrane potential as $\Delta\psi = \psi_{cis} - \psi_{trans}$, where $\psi_{trans} = 0$ mV. The cis side is the side to which PA_{63} and LF_N are added.
11. Single-letter abbreviations for the amino acid residues are as follows: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr.
12. B. R. Sellman, S. Nassi, R. J. Collier, *J. Biol. Chem.* **276**, 8371 (2001).
13. Mutation of several other sites in domain 2 were also shown to block translocation, but these prevented pore formation (*12*, *25*).
14. B. Hille, *J. Gen. Physiol.* **51**, 199 (1968).
15. S. Zhang, A. Finkelstein, R. J. Collier, *Proc. Natl. Acad. Sci. U.S.A.* **101**, 16756 (2004).
16. R. O. Blaustein, A. Finkelstein, *J. Gen. Physiol.* **96**, 905 (1990).
17. N. Zacharias, D. A. Dougherty, *Trends Pharmacol. Sci.* **23**, 281 (2002).
18. L. Wesson, D. Eisenberg, *Protein Sci.* **1**, 227 (1992).
19. F. Orlik, B. Schiffler, R. Benz, *Biophys. J.* **88**, 1715 (2005).
20. W. Kauzmann, *Adv. Protein Chem.* **14**, 1 (1959).
21. M. Zhou, J. H. Morais-Cabral, S. Mann, R. MacKinnon, *Nature* **411**, 657 (2001).
22. D. A. Doyle *et al.*, *Science* **280**, 69 (1998).
23. B. Van den Berg *et al.*, *Nature* **427**, 36 (2004).
24. T. Schirmer, T. A. Keller, Y. F. Wang, J. P. Rosenbusch, *Science* **267**, 512 (1995).

25. M. Mourez *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **100**, 13803 (2003).
26. B. A. Krantz, R. A. Melnyk, R. J. Collier, A. Finkelstein, unpublished data.
27. D. B. Lacy, D. J. Wigelsworth, R. A. Melnyk, S. C. Harrison, R. J. Collier, *Proc. Natl. Acad. Sci. U.S.A.* **101**, 13147 (2004).
28. Materials and methods are available as supporting material on *Science* Online.
29. Translocation of LF_N (and full-length LF) was greatly accelerated when the pH on the trans side was greater than that on the cis side (*26*).
30. R.J.C. is cofounder, member of the scientific advisory board, and equity holder in PharmAthene, Inc., a startup company that investigates countermeasures against anthrax and other bioterrorism agents. We thank K. J. Oh for EPR data acquisition; R. Ross at the New England Research Center of Excellence Biomolecule Production Core as well as R. Pimental, L. Greene, and H. Lin for their assistance in purifying and generating PA mutants; and W. Hubbell, T. Sosnick, and A. Johnson for useful discussions. This work was supported by a National Research Service Award fellowship, AI062204 (B.A.K.), and NIH grants, AI022021 (R.J.C.) and GM29210 (A.F.).

Supporting Online Material
www.sciencemag.org/cgi/content/full/309/5735/777/DC1
Materials and Methods
SOM Text
Figs. S1 to S3
Tables S1 and S2
References and Notes

11 April 2005; accepted 2 June 2005
10.1126/science.1113380

Genetic Tracing Shows Segregation of Taste Neuronal Circuitries for Bitter and Sweet

Makoto Sugita* and Yoshiki Shiba

The recent discovery of mammalian bitter, sweet, and umami taste receptors indicates how the different taste qualities are encoded at the periphery. However, taste representations in the brain remain elusive. We used a genetic approach to visualize the neuronal circuitries of bitter and sweet tastes in mice to gain insight into how taste recognition is accomplished in the brain. By selectively expressing a transsynaptic tracer in either bitter- or sweet and/or umami–responsive taste receptor cells, and by comparing the locations of the tracer-labeled neurons in the brain, our data revealed the potential neuronal bases that underlie discrimination of bitter versus sweet.

The gustatory system is primarily devoted to a quality check of food, while at the same time detecting nutrients and avoiding toxic substances. The initial step in taste perception takes place at the apical end of taste receptor cells, tightly packed into taste buds of the oral epithelium. The cells express taste receptors, which are responsible for detecting and distinguishing among sweet, bitter, salty, sour, and umami stimuli (*1*). In mammals, bitter and sweet and/or umami are the two main taste modalities evoking aversion and attraction, respectively. Humans also express pleasure for sweet taste but displeasure for bitter taste. On the other hand, mammals learn to reject a tastant if this tastant is associated with subsequent visceral malaise (*2*). Therefore, it is likely that the mammalian gustatory system is an excellent system to address the question of how emotion interacts with cognition and memory. To decipher rationally the underlying molecular, cellular, and system mechanisms, it is first necessary to understand and to compare precisely the contrastive neuronal circuitries that process and integrate the information of aversive and attractive taste modalities in the whole brain.

Department of Physiology and Oral Physiology, Graduate School of Biomedical Sciences, Hiroshima University, Kasumi 1-2-3, Minami-ku, Hiroshima 734-8553, Japan.

*To whom correspondence should be addressed. E-mail: sugisan@hiroshima-u.ac.jp

Bitter tastants are detected by members of a family of 30 different G protein–coupled receptors (GPCRs), the T2Rs (*3*–*5*). Sweet and umami tastes are substantially mediated by a small family of three GPCRs (T1R1, T1R2, and T1R3). T1R2 and T1R3 combine to function as a sweet receptor, whereas T1R1 and T1R3 form the umami receptor, which detects glutamate (*6*, *7*). Sweet, umami, and bitter receptors appear to be expressed in distinct populations of taste cells that operate independently of each other to trigger taste recognition (*6*, *8*–*10*). The receptor cells are innervated by afferent fibers that transmit information to the gustatory cortex through synapses in the brain stem and thalamus (*11*). How is taste information processed in the central nervous system, while it is discriminated and as it evokes the emotional and behavioral responses such as aversion and attraction? We applied a genetic approach to visualize the neuronal circuitries of bitter and sweet-umami taste by using the taste receptor genes and the plant lectin WGA as molecular tools. Injected lectin proteins are an effective tracer for transsynaptically delineating the wiring patterns in the central nervous system (*12*–*14*). Furthermore, the genetic approach using the WGA transgene, expressed under the control of specific promoter elements, is a powerful tool for tracing selective and functional neuronal circuitries originating from a specific type of neuron (*15*, *16*).

We prepared transgenic mice in which the transsynaptic tracer WGA, C-terminally truncated and fused by the fluorescent protein (tWGA-DsRed), was coexpressed with selected taste receptors. The transgene for tracing the bitter taste neuronal circuitries is shown in Fig. 1A. We selected the promoter element of the mT2R5 gene, reported as a receptor for cycloheximide (*5*), to drive tWGA-DsRed expression in bitter receptor–expressing cells. In phospholipase Cβ2 (PLCβ2)–deficient mice, which lack sweet, amino acid, and bitter taste reception, the PLCβ2 transgene, expressed under the control of the mT2R5 promoter, rescued the response to multiple bitter compounds, but not to sweet or amino acid taste (*8*, *10*). The findings support not only that taste receptor cells are not broadly tuned across these modalities, but also that the mT2R5-expressing cells coexpress the multiple T2Rs and are capable of responding to a broad array of bitter compounds. The transgene was constructed by connecting the promoter element of mT2R5, the fusion constructs of mT2R5 with green fluorescent protein (mT2R5-GFP) and tWGA-DsRed, which intercalated the internal ribosome entry site (IRES), and the polyadenylation signal (Fig. 1A). To test whether a part of the transgene works, Cos7 cells and HEK293 cells were transiently transfected with the construct, in which the upstream region of mT2R5 was replaced by the cytomegalovirus (CMV) promoter (Fig. 1B). The fusion proteins of mT2R5-GFP and tWGA-DsRed were expressed in these cells (Fig. 1B), which suggested that the transgene produces a bicistronic mRNA from which mT2R5-GFP and tWGA-DsRed are independently translated. Furthermore, the tWGA-DsRed fusion protein was associated with the intracellular granule–like structures of cultured cells. The mT2R5-GFP fusion protein was localized in the cell surface membrane (Fig. 1B). In addition, fura-2 calcium imaging of HEK293 cells, cotransfected with T2Rs-GFP and Gα15, revealed that fusion of GFP to the C terminus of T2Rs does not affect the receptor function (*17*).

First, to verify the expression patterns of the endogenous mT2R5 and the transgene in the transgenic mice (mT2R5-WGA), we performed in situ hybridization using the 3′ untranslated region of mT2R5 and the GFP-coding region as probes for double-label fluorescent detection. The cells expressing the

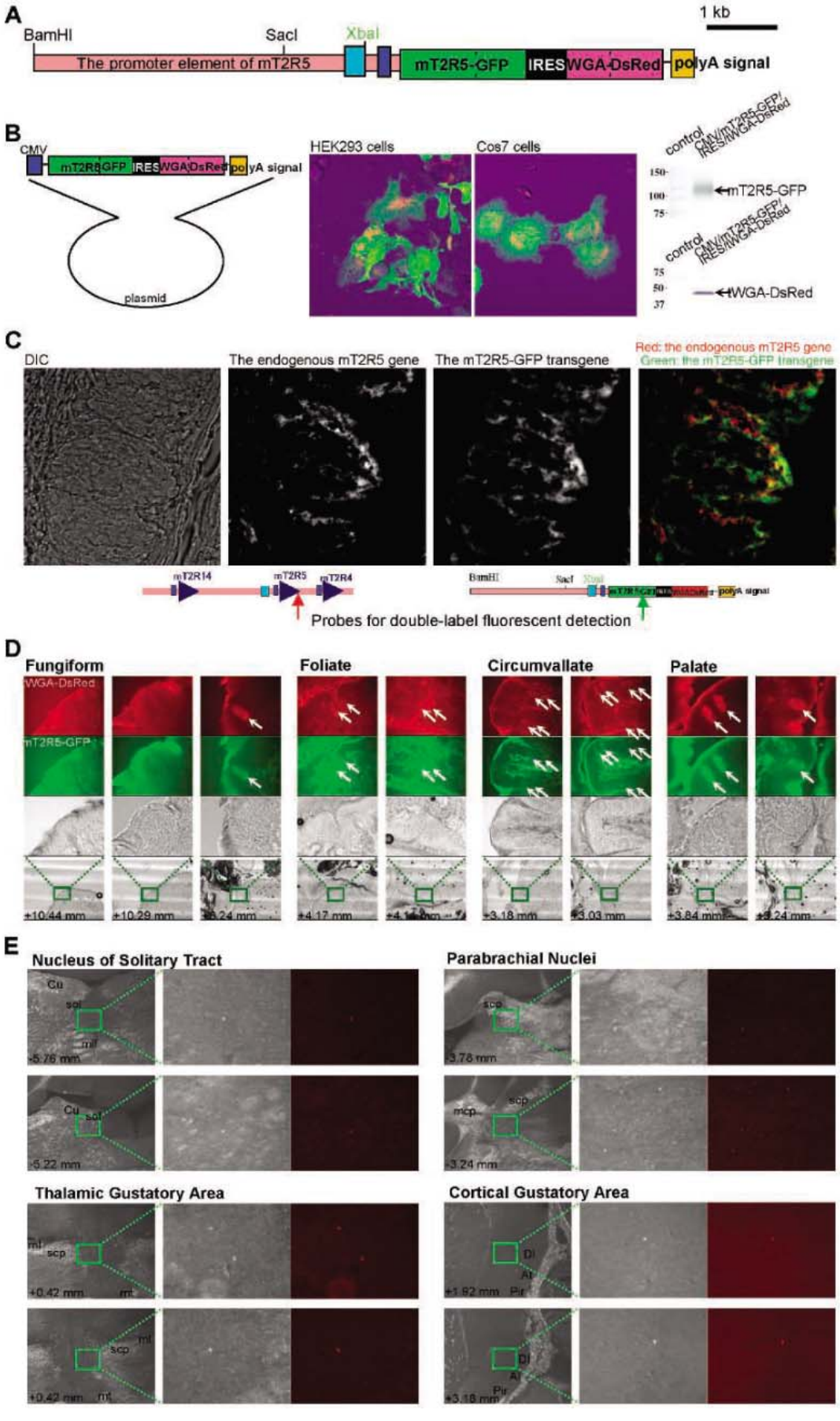


Fig. 1. Generation of the mT2R5-WGA mouse to visualize bitter taste neuronal circuitries. (**A**) Schematic diagram indicating the structure of the transgene to trace bitter taste neuronal circuitries. Blue boxes represent the homologous regions, found in the 5′ upstream sequences of mT2R5 and human T2R10. (**B**) Expression of mT2R5-GFP and tWGA-DsRed in cultured cells. Subcellular distribution of mT2R5-GFP and tWGA-DsRed, transiently expressed under the control of the CMV promoter in Cos7 cells and HEK293 cells, was directly visualized by the GFP and DsRed fluorescence. The expression levels of mT2R5-GFP and tWGA-DsRed were monitored with immunoblotting by using antibodies against GFP and WGA, respectively. (**C**) In situ hybridization demonstrated concordance in the expression pattern of the endogenous mT2R5 gene (red) and the transgene (green). (**D**) Direct fluorescence detection of mT2R5-GFP and tWGA-DsRed, expressed in taste receptor cells. Arrows indicate the transgene-expressing taste buds. (**E**) Spatial distribution of tWGA-DsRed in coronal sections of the mT2R5-WGA mouse brain, clarified by direct fluorescence detection (right). Dark-field images at the same magnification (middle) and at the lower magnification (left) were also shown. The distance to the posterior end of the fasciculus retroflexus (pfr) was calculated and denoted in each section.

endogenous mT2R5 receptor also expressed the transgene (Fig. 1C). Next, we analyzed the expression patterns of the transgene at a protein level by direct fluorescence detection of mT2R5-GFP and tWGA-DsRed in taste buds. mT2R5-GFP and tWGA-DsRed were rarely expressed in fungiform papillae. The few fungiform taste buds that express both mT2R5-GFP and tWGA-DsRed appear clustered at the posterior region of the front half of the tongue surface (Fig. 1D). Taste buds expressing mT2R5-GFP and tWGA-DsRed were detected in circumvallate papillae, foliate papillae, and the palate epithelium (Fig. 1D), consistent with the expression patterns of T2Rs (*3*, *4*).

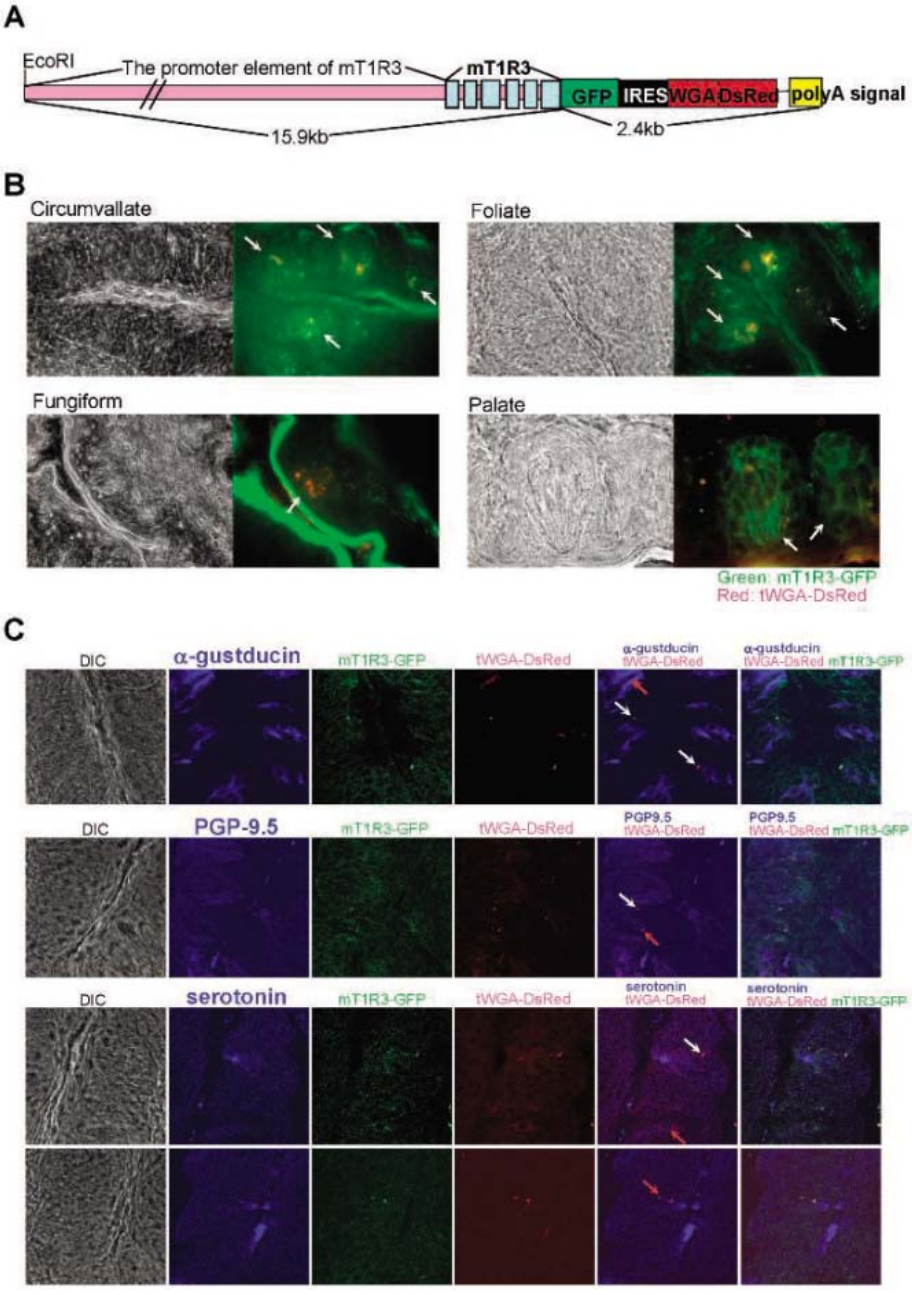


Fig. 2. Generation of the mT1R3-WGA mouse to visualize sweet-umami taste neuronal circuitries. (**A**) Schematic diagram indicating the structure of the transgene to trace sweet-umami taste neuronal circuitries. (**B**) Direct fluorescence detection of mT1R3-GFP and tWGA-DsRed, expressed in taste receptor cells. Arrows indicate the transgene-expressing taste buds. (**C**) Confocal images showing localization of mT1R3-GFP, tWGA-DsRed, α-gustducin, PGP-9.5, and 5-HT in taste buds of mT1R3-WGA mice. Location of mT1R3-GFP and tWGA-DsRed was clarified by detecting GFP and DsRed fluorescence. Locations of α-gustducin, PGP-9.5, and 5-HT were detected using the primary antibodies against those proteins and Alexa-633–conjugated secondary antibodies. However, Alexa-633 fluorescence was replaced by the pseudocolor blue and overlaid. White arrows indicate the mT1R3-GFP– and tWGA-DsRed–expressing cells without immunoreactivity for α-gustducin, PGP-9.5, or 5-HT. Red arrows indicate the mT1R3-GFP– and tWGA-DsRed–expressing cells with immunoreactivity for α-gustducin, PGP-9.5, or 5-HT.

In the mT2R5-WGA mouse brain, we analyzed the spatial distribution of tWGA-DsRed, which is expressed in the specific taste receptor cells and transferred to the neurons. Anatomical and physiological data have shown the nuclear relays and their connecting pathways in the central gustatory system (*11*–*14*, *18*–*20*), although the exact dimensions and internal organization for each taste modality remain unclear. Here, tWGA-DsRed was located in the posterior part of the solitary tract nuclei (Sol), the pontine parabrachial nuclei (PB), the thalamic gustatory area (Gus), and the gustatory cortex (DI) (Fig. 1E). Labeling in the most posterior part of the solitary tract nuclei might be associated with a projection from T2R-expressing cells of the gut. DsRed fluorescence was also observed in the amygdala and the olfactory cortex (fig. S1C). Neither mT2R5-GFP fluorescence nor in situ hybridized tWGA mRNA was detected in those parts of the brain.

To compare the neuronal circuitries of bitter and sweet-umami taste, we selectively expressed mT1R3-GFP and tWGA-DsRed in sweet-umami–responsive taste cells (mT1R3-WGA) under the control of the specific promoter element of the sweet-umami taste receptor mT1R3. The transgene is shown in Fig. 2A. The identical promoter element drives the transgene expression in perfect concordance with the endogenous mT1R3 gene (*6*). In mT1R3-WGA mice, mT1R3-GFP and tWGA-DsRed were coexpressed in subsets of taste receptor cells located in fungiform, foliate, circumvallate, and Geschmackstreifen (taste stripes) taste buds (Fig. 2B). tWGA-DsRed was located in the anterior part of Sol, PB, Gus, and DI (fig. S2). To characterize the cell type of the transgene-expressing cells in taste buds, we examined the cellular distribution of tWGA-DsRed in comparison with α-gustducin, ubiquitin carboxyl terminal hydrolase (PGP-9.5), and serotonin (5-HT). Taste cells are characterized by expressions of these marker proteins and are divided into three classes (types I, II, and III) (*21*). Both mT1R3-GFP and tWGA-DsRed were detected in taste cells with and without α-gustducin (Fig. 2C). Furthermore, mT1R3-GFP and tWGA-DsRed were coexpressed in subsets of both PGP-9.5–immunoreactive cells and 5-HT–immunoreactive cells (Fig. 2C). These results suggest that, at least, subsets of both the type II cells and type III cells with obvious synapses onto afferent nerve fibers expressed mT1R3-GFP and tWGA-DsRed at a protein level.

Then, we focused on the spatial distribution of tWGA-DsRed–labeled neurons in the brain. To characterize the precise distribution of

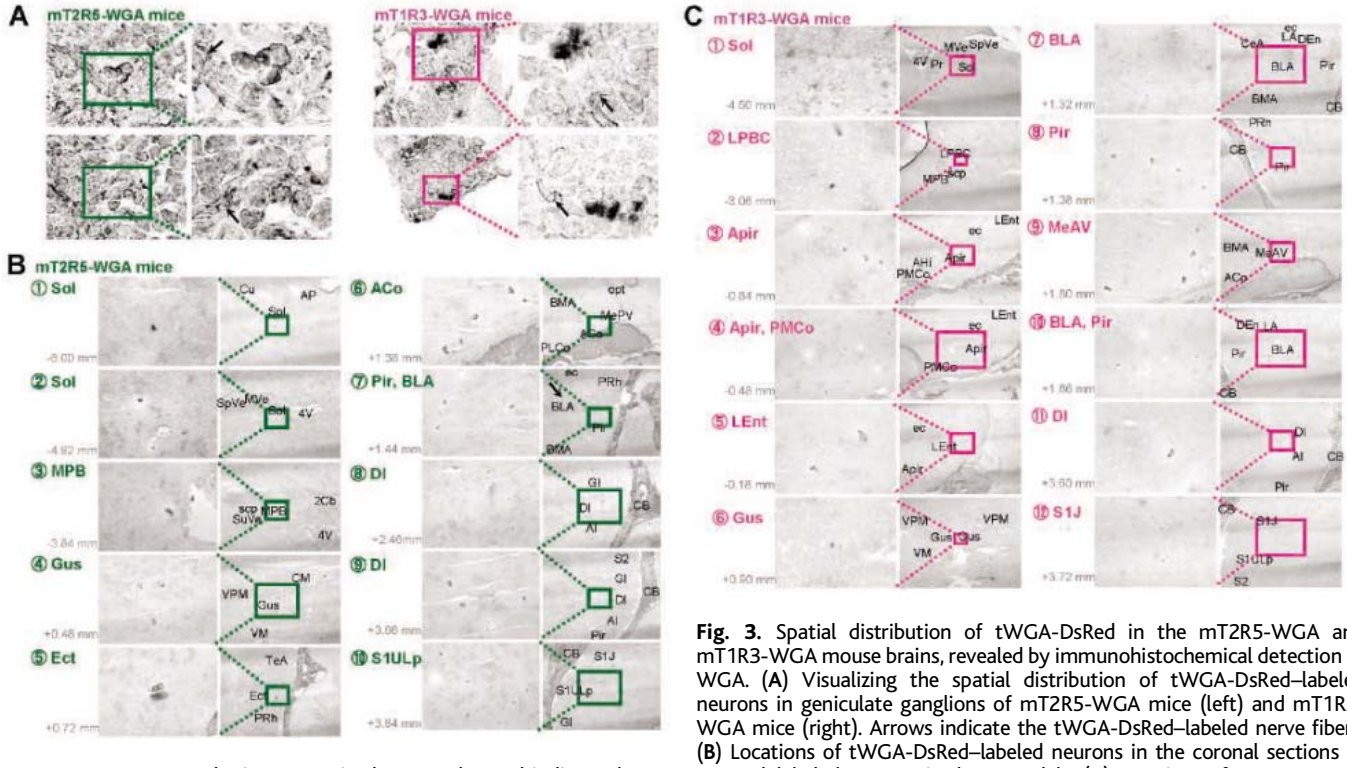


Fig. 3. Spatial distribution of tWGA-DsRed in the mT2R5-WGA and mT1R3-WGA mouse brains, revealed by immunohistochemical detection of WGA. (**A**) Visualizing the spatial distribution of tWGA-DsRed–labeled neurons in geniculate ganglions of mT2R5-WGA mice (left) and mT1R3-WGA mice (right). Arrows indicate the tWGA-DsRed–labeled nerve fibers. (**B**) Locations of tWGA-DsRed–labeled neurons in the coronal sections of the mT2R5-WGA mouse brain. Arrows in the seventh panel indicate the tWGA-DsRed–labeled neurons in the amygdala. (**C**) Locations of tWGA-DsRed–labeled neurons in the coronal sections of the mT1R3-WGA mouse brain. The distance to pfr was calculated and denoted in each section.

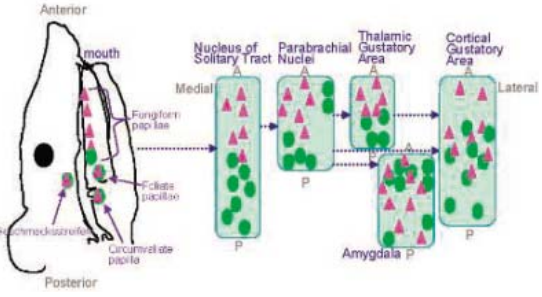


Fig. 4. Schematic representation of the spatial distribution of tWGA-DsRed–labeled cells in mT2R5 and mT1R3 mice. Locations of tWGA-DsRed–labeled cells in the mouth, the solitary tract nucleus, the pontine parabrachial nucleus, the thalamic gustatory area, the amygdala, and the cortical gustatory area are plotted using green circles for mT2R5-WGA mice and red triangles for mT1R3-WGA mice.

tWGA-DsRed–labeled neurons in mT1R3-WGA mice in comparison with mT2R5-WGA mice and to permanently and repetitively observe the labeled neuron in the brain, we performed immunohistochemical detection of WGA in the brain. First, in geniculate ganglion neurons, which provide innervation to taste buds via the corda tympani nerve branch (*11*), the clusters of tWGA-DsRed–labeled neurons were dispersed in serial sections of ganglions, isolated from both the mT1R3-WGA and mT2R5-WGA mice (Fig. 3A). WGA immunoreactivity was also detected in subsets of nerve fibers (Fig. 3A). However, we could not deduce the differences in the spatial distributions of tWGA-DsRed–labeled geniculate ganglion neurons between mT1R3-WGA and mT2R5-WGA mice. In higher brain centers, segregation of inputs from mT1R3 and mT2R5 was revealed. tWGA-DsRed–labeled neurons are located in the coronal sections of the transgenic mouse brains (Fig. 3, B and C). In the mT2R5-WGA mouse brain, WGA immunoreactivity was detected in the broad but specific regions including Sol, the medial PB (MPB), Gus, and DI. tWGA-DsRed–labeled neurons were also observed in the amygdala (ACo, BLA), the olfactory cortex (Pir), and the primary somatosensory cortex (S1ULp) (Fig. 3B). Similarly, in the mT1R3-WGA mouse brain, tWGA-DsRed–labeled neurons were widely but restrictedly distributed to Sol, PB (MPB, LPBD, LPBC), Gus, DI, amygdala (APir, PMCo, BLA, MeAV), the olfactory cortex (LEnt, Pir), and the primary somatosensory cortex (S1J) (Fig. 3C). Differences in the tWGA-DsRed–labeled patterns of the two strains are detailed in online supporting text and fig. S3.

It is noteworthy that the positions of labeled neuronal clusters in Sol, PB, Gus, DI, and the amygdala revealed similar patterns in different individuals in each strain (n = 5 and 7 for mT1R3-WGA mice and mT2R5-WGA mice, respectively), which show that the gustatory neurons dispersed in those regions were organized with sweet inputs rostral and with bitter inputs caudal, except for bitter inputs into ELPB and EMPB and the complex inputs into the amygdala (Fig. 4 and fig. S3). Although densely labeled neurons were scattered in the broad areas, most of the clusters appeared to be bilaterally asymmetrical in the left and right hemispheres of both the mT1R3-WGA and mT2R5-WGA mouse brains (fig. S3, A, E, and F). Judging from the distributional patterns of densely labeled neurons, inputs from mT2R5 and mT1R3 may be concentrated on the small size of the gustatory relays in the brain stem, the thalamus, and the cortex. However, it is possible that efficiency of tWGA-DsRed transfer might not parallel the strength of synaptic transmission, and that the neurons that contained only a small amount of tracer and were not detected might also relay taste information.

Thus, the two strains of mice, expressing a tracer transgene in specific taste cells, enabled us to map connections formed by small subsets of neurons, which process and integrate the information of bitter taste, separated from sweet-umami taste. The mapping may be influenced by a certain equilibrium among the efficiencies of biogenesis, transport, and degradation of the tracer, which may vary depending on the developmental stages of the mouse brain and peripheral taste systems. Nevertheless, these transgenic mice can reveal the molecular aspects underlying the construction and refine-

ment of taste neuronal circuitries, especially in combination with the gene-targeted mutant mice for key molecules.

References and Notes

1. B. Lindemann, *Nature* **413**, 219 (2001).
2. D. E. Berman, Y. Dudai, *Science* **291**, 2417 (2001).
3. H. Matsunami, J. P. Montmayeur, L. B. Buck, *Nature* **404**, 601 (2000).
4. E. Adler *et al.*, *Cell* **100**, 693 (2000).
5. J. Chandrashekar *et al.*, *Cell* **100**, 703 (2000).
6. G. Nelson *et al.*, *Cell* **106**, 381 (2001).
7. G. Nelson *et al.*, *Nature* **416**, 199 (2002).
8. Y. Zhang *et al.*, *Cell* **112**, 293 (2003).
9. G. Q. Zhao *et al.*, *Cell* **115**, 255 (2003).
10. K. L. Mueller *et al.*, *Nature* **434**, 225 (2005).
11. R. Norgren, in *The Rat Nervous System*, G. Paxinos, Ed. (Academic Press, San Diego, 1995), pp. 751–771.
12. H. Herbert, M. M. Moga, C. B. Saper, *J. Comp. Neurol.* **293**, 540 (1990).
13. J. F. Bernard, M. Alden, J. M. Besson, *J. Comp. Neurol.* **329**, 201 (1993).
14. H. Bester, L. Bourgeais, L. Villanueva, J. M. Besson, J. F. Bernard, *J. Comp. Neurol.* **405**, 421 (1999).
15. Y. Yoshihara *et al.*, *Neuron* **22**, 33 (1999).
16. Z. Zou, L. F. Horowitz, J.-P. Montmayeur, S. Snapper, L. B. Buck, *Nature* **414**, 173 (2001).
17. M. Sugita, unpublished results.
18. R. B. Hamilton, R. Norgren, *J. Comp. Neurol.* **222**, 560 (1984).
19. R. Norgren, *J. Comp. Neurol.* **166**, 17 (1976).
20. G. V. Allen, C. B. Saper, K. M. Hurley, D. F. Cechetto, *J. Comp. Neurol.* **311**, 1 (1991).
21. C. L. Yee, K. R. Jones, T. E. Finger, *J. Comp. Neurol.* **459**, 15 (2003).

22. We are grateful to the members in Research Facilities for Laboratory Animal Science, Natural Science Center for Basic Research and Development, Hiroshima University, for supporting animal experiment. We thank H. Ohishi for expert help in calcium imaging, Y. Yoshihara for truncated WGA, S. Offermanns and M. I. Simon for Gα15, and P. Mombaerts for ETLpA-/LTNL. This research was supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science, Sports, and Culture of Japan, and the Sumitomo Foundation (to M.S.).

Supporting Online Material

www.sciencemag.org/cgi/content/full/309/5735/781/DC1
Materials and Methods
SOM Text
Figs. S1 to S3
References and Notes

7 February 2005; accepted 10 June 2005
10.1126/science.1110787

The Role of Social Groups in the Persistence of Learned Fear

Andreas Olsson,[1] Jeffrey P. Ebert,[3] Mahzarin R. Banaji,[3] Elizabeth A. Phelps[1,2]*

Classical fear conditioning investigates how animals learn to associate environmental stimuli with an aversive event. We examined how the mechanisms of fear conditioning apply when humans learn to associate social ingroup and outgroup members with a fearful event, with the goal of advancing our understanding of basic learning theory and social group interaction. Primates more readily associate stimuli from certain fear-relevant natural categories, such as snakes, with a negative outcome relative to stimuli from fear-irrelevant categories, such as birds. We assessed whether this bias in fear conditioning extends to social groups defined by race. Our results indicate that individuals from a racial group other than one's own are more readily associated with an aversive stimulus than individuals of one's own race, among both white and black Americans. This prepared fear response might be reduced by close, positive interracial contact.

In classical fear conditioning, a neutral stimulus acquires aversive properties by virtue of simply being paired in time with an aversive event. In general, research on classical conditioning has not emphasized differences between classes of stimuli, instead focusing on principles that apply across different kinds of stimuli (*1*). One important exception is research on selective, or prepared, aversive learning. For both humans (*2*, *3*) and nonhuman primates (*4*), stimuli from certain fear-relevant natural categories, such as snakes and spiders, are more readily associated with aversive events than stimuli from fear-irrelevant categories, such as birds and butterflies (*5*). We investigated whether prepared learning can be extended to fear associated with members of another, as compared with one's own, racial group. Recent studies have observed that race bias and fear conditioning may indeed rely on overlapping neural systems (*6*–*8*), suggesting a potential link in mechanism and the opportunity to use classical fear conditioning as a model for aversive learning in a socio-cultural context (*9*, *10*).

We assessed whether individuals of another race are more readily associated with an aversive stimulus than individuals of one's own race, and whether these effects may be moderated by attitudes, beliefs, or contact with members of the racial outgroup. In humans, prepared fear learning has been most consistently demonstrated as a persistence in the learned fear response to fear-relevant conditioned stimuli (*11*). If representations of racial outgroup but not ingroup members act like prepared stimuli, we would expect that fear responses acquired to outgroup faces would persist during extinction relative to fear responses acquired to ingroup faces. To test this prediction, we conducted two experiments whose procedures differed only with respect to the stimuli used (*12*). The first was designed to recreate the standard preparedness effect for traditional fear-relevant stimuli, and the second was designed to test this effect in the context of human social groups defined by race.

Experiment 1 presented subjects with images of two typically used exemplars of fear-relevant (a snake and a spider) and fear-irrelevant (a bird and a butterfly) stimuli in order to verify that the experimental manipulations effectively replicated previous findings. Experiment 2 presented black and white American participants images of faces of two black and two white unfamiliar male individuals with neutral expressions. During fear acquisition, one stimulus (the reinforced conditioned stimulus, CS+) from each stimulus category was paired with a mild electric shock (the unconditioned stimulus, UCS), which was individually adjusted to be perceived as uncomfortable, but not painful. The other stimulus from each category (the unreinforced conditioned stimulus, CS–) was presented without shock. Each presentation of a CS was 6 s, and the UCS co-terminated with each presentation of a CS+ during acquisition. During the extinction phase that followed, no shocks were administered. Skin conductance responses (SCRs) were measured during both acquisition and extinction trials. The conditioned fear response (CR) was assessed as the differential SCR, that is, the SCR to the CS+ minus the SCR to the CS– from the same stimulus category, thereby reducing preexisting differences in the emotional salience of stimulus categories as a confounding variable. In experiment 2, after completion of the extinction phase, subjects completed implicit and explicit measures of race attitudes and stereotypes, as well as self-report measures of contact with racial ingroup and outgroup members. The within-subject design of the conditioning paradigm allowed us to compute a relative measure of conditioning race bias that could be linked to each participant's relative measures of race attitudes, stereotypes, and intergroup contact.

The mean differential SCRs during acquisition and extinction in experiment 1 are presented in Fig. 1A. During acquisition, there

[1]Department of Psychology and [2]Center for Neural Science, New York University, 6 Washington Place, New York, NY 10003, USA. [3]Department of Psychology, Harvard University, 33 Kirkland Street, Cambridge, MA 02138, USA.

*To whom correspondence should be addressed. E-mail: liz.phelps@nyu.edu

was a significantly greater SCR to the CS+ compared with the CS– for both fear-relevant [$t(16) = 5.81$, $P < 0.0001$] and fear-irrelevant [$t(16) = 4.24$, $P < 0.001$] stimuli, indicating acquisition of a CR to both classes of stimuli. As predicted, in the extinction phase, subjects' CRs to snakes and spiders failed to fully extinguish [$t(16) = 2.81$, $P < 0.05$], whereas their CRs to birds and butterflies did [$t(16) = 0.98$, not significant (NS)]. These results replicate earlier results showing a greater persistence of fear learning for fear-relevant than fear-irrelevant conditioned stimuli (*3*, *11*).

The mean differential SCRs during acquisition and extinction to human faces from social groups in experiment 2 are plotted in Fig. 1B. Overall, there was a greater SCR for the CS+ versus the CS– for both racial ingroup [$t(72) = 5.28$, $P < 0.0001$] and outgroup [$t(72) = 8.10$, $P < 0.0001$] faces during acquisition, demonstrating a CR to both. In extinction, there was a persistent, significant CR to racial outgroup faces [$t(72) = 3.87$, $P < 0.0001$], whereas the CR to ingroup races was fully extinguished [$t(72) = -0.29$, NS]. This persistence of fear learning during extinction for outgroup members mirrors the pattern observed for snakes and spiders in experiment 1 (*13*).

This prepared learning effect is displayed separately for white (Fig. 2A) and black American (Fig. 2B) participants. White participants displayed a greater SCR to the CS+ versus the CS– for both black [$t(35) = 6.03$, $P < 0.0001$] and white [$t(35) = 3.96$, $P < 0.001$] faces during acquisition. As predicted, white participants' CRs to black faces did not fully extinguish [$t(35) = 2.85$, $P < 0.01$], whereas their CRs to white faces did [$t(35) = –0.91$, NS]. During acquisition, black participants displayed a greater SCR to the CS+ versus the CS– for both black [$t(36) = 3.52$, $P < 0.01$] and white [$t(36) = 5.44$, $P < 0.0001$] faces, indicating acquisition of a CR. Following the same pattern of outgroup bias exhibited by the white participants, black participants' CRs to white faces did not fully extinguish [$t(36) = 2.59$, $P < 0.05$], whereas their CRs to black faces did [$t(36) = 1.10$, NS].

The extinction data show that unfamiliar members of a racial outgroup can serve as prepared stimuli in a fear-learning situation. These data concur with studies demonstrating that primates selectively associate stimuli from relevant natural categories with an aversive outcome (*11*). Our findings are also consistent with imaging data linking race bias in evaluating others with subcortical brain systems that mediate fear learning across species (*6*–*8*). The propensity to associate aversive events with outgroup members could lead to more negative evaluations of the outgroup, given otherwise equivalent properties of ingroup and outgroup members. In this respect, the outgroup preparedness finding belongs with other psychological mechanisms that have been identified as contributing to the genesis and maintenance of racial prejudice, especially implicit or less conscious forms of it (*14*–*17*).

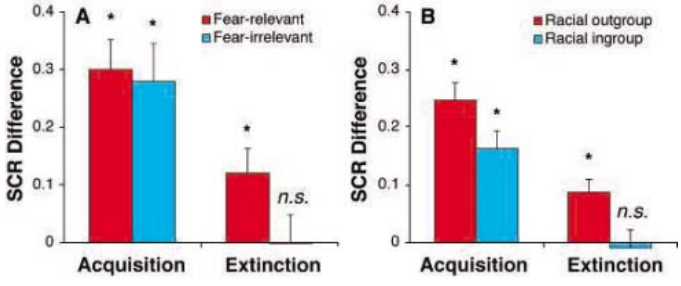


Fig. 1. Mean conditioned response, CR (scaled SCR difference), as a function of stimulus category. Error bars indicate standard errors. Asterisks indicate a statistically significant CR, and "n.s." indicates the CR is not significantly different from zero. (**A**) Experiment 1: there was a CR to both fear-relevant and fear-irrelevant stimuli during acquisition. Only CRs to fear-relevant stimuli resisted extinction. (**B**) Experiment 2: there was a CR to both outgroup and ingroup faces during acquisition. Mimicking the response pattern observed in experiment 1, only CRs to outgroup faces resisted extinction.

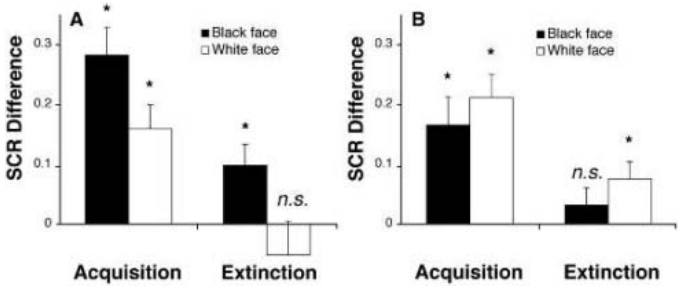


Fig. 2. Mean conditioned response, CR (scaled SCR difference), as a function of race category. Error bars indicate standard errors. Asterisks indicate a statistically significant CR, and "n.s." indicates the CR is not significantly different from zero. (**A**) White participants acquired a CR to both black and white faces, but only their CR to black faces resisted extinction. (**B**) Black participants acquired a CR to both black and white faces, but only their CR to white faces resisted extinction.

We examined whether the conditioning bias to outgroup faces was moderated by attitudes and beliefs about the outgroup or the amount of contact with outgroup members. The only measure found to significantly moderate the conditioning bias was interracial dating [Supporting Online Material (SOM) Text]. Specifically, the conditioning bias to outgroup faces was negatively correlated with the reported number of outgroup, relative to ingroup, romantic partners [$r(68) = –0.29$, $P < 0.05$]. In other words, the conditioning bias to fear racial outgroup members was attenuated among those with more interracial dating experience, consistent with a substantial body of research demonstrating that positive intergroup contact reduces negativity toward outgroups (*18*). Because this is a correlational analysis, this finding could instead indicate that a third variable highly correlated with interracial dating is causally important in the reduction of outgroup preparedness or that those individuals strongest in outgroup preparedness are less likely to date interracially. In this sample, more black participants reported interracial dating (51%) than white participants (28%). Figure S1 and table S4 illustrate the similarity of conditioning effects for black and white participants who had only same-race dating experiences.

What remains to be explained is why individuals associate racial outgroup members more easily with an aversive stimulus, and to this end previous research on prepared fear learning allows a challenge to existing ways of thinking about social learning. Demonstrations of prepared learning have typically been taken as evidence for biologically evolved learning mechanisms that treat certain natural categories of stimuli as prepared to be associated with an aversive outcome (*19*, *20*). This interpretation has received support from a range of findings. Conditioned responses to fear-relevant stimuli are especially insensitive to cognitive manipulations: Instructed extinction fails (*21*), and conditioned responses are elicited even when conditioned stimuli are presented without conscious awareness (*22*). In addition, the prepared learning effect does not extend to most culturally defined fear-relevant stimuli, such as broken electrical outlets and some representations of weapons (*2*, *23*), suggesting that fear relevance alone does not mediate this effect. However, at least one study reports that a fear-relevant cultural artifact (e.g., a pointed gun), when paired with a pertinent UCS (e.g., a loud noise), can produce a resistance to extinction that is comparable to that elicited by natural categories of fear-relevant stimuli (*24*). This result suggests that, under certain circumstances, cultural learning can imbue a stimulus with qualities that engage similar learning mechanisms as do spiders and snakes.

The evolutionary interpretation for the results of experiment 1 is relatively straight-

forward: Modern primates are predisposed to learn to fear spiders and snakes because such preparedness conferred a selective advantage to our ancestors over conspecifics that were not thus prepared (*11*). A similar argument has previously been made for the superior conditioning effect observed to angry in comparison with happy faces, emphasizing the evolutionary relevance of the face as a means of signaling threat (*25*). The evolutionary interpretation for the racial outgroup bias found in experiment 2 is more nuanced. The differentiation of *Homo sapiens* into what modern humans recognize as distinct races occurred relatively recently in human evolutionary history, by some estimates within the past 100,000 to 200,000 years (*26*). Critically, it is believed that this differentiation occurred precisely because of the mass migration and consequent geographic isolation of different human lineages, meaning that natural selection could not have specifically prepared whites to fear blacks and blacks to fear whites. However, humans might have evolved a more general preparedness to fear others who were dissimilar to them or who otherwise appeared not to belong to their social group because such individuals were more likely to pose a threat (*27*, *28*). If a general preparedness to fear dissimilar others did indeed evolve, then present-day members of another race, with their physical differences and common categorization as belonging to an outgroup, could activate such a mechanism and produce the robust conditioning effect observed in experiment 2.

In other words, because of its relatively recent emergence as an important dimension in human social interaction, race inherently cannot be the basis of the outgroup preparedness result. Instead, it is likely that sociocultural learning about the identity and qualities of outgroups is what provides the basis for the greater persistence of fear conditioning involving members of another group. Most notably, individuals acquire negative beliefs about outgroups according to their local cultures, and few reach adulthood without considerable knowledge of these prejudices and stereotypes (*14*, *29*, *30*). It is plausible that repeated exposure to information about outgroups might prepare individuals to fear newly encountered outgroup members.

Further research will pinpoint the generality and the interpretation of the outgroup bias in aversive conditioning. For now, our finding that close, intergroup contact may reduce this bias suggests that individual experiences can play a moderating role. Millennia of natural selection and a lifetime of social learning may predispose humans to fear those who seem different from them; however, developing relationships with these different others may be one factor that weakens this otherwise strong predisposition.

References and Notes

1. I. P. Pavlov, *Conditioned Reflexes* (Oxford Univ. Press, Oxford, 1927).
2. E. W. Cook, R. L. Hodes, P. J. Lang, *J. Abnorm. Psychol.* **95**, 195 (1986).
3. A. Öhman, M. Fredrikson, K. Hugdahl, P. A. Rimmö, *J. Exp. Psychol. Gen.* **103**, 313 (1976).
4. S. Mineka, M. Davidson, M. Cook, R. Keir, *J. Abnorm. Psychol.* **93**, 355 (1984).
5. In humans, a superior conditioning effect has also been demonstrated with angry compared with happy faces [see (*25*) for a review on faces as conditioned stimuli].
6. E. A. Phelps *et al.*, *J. Cogn. Neurosci.* **12**, 729 (2000).
7. A. J. Hart *et al.*, *Neuroreport* **11**, 2351 (2000).
8. W. A. Cunningham *et al.*, *Psychol. Sci.* **15**, 806 (2004).
9. E. A. Phelps *et al.*, *Nat. Neurosci.* **4**, 437 (2001).
10. A. Olsson, E. A. Phelps, *Psychol. Sci.* **15**, 822 (2004).
11. A. Öhman, S. Mineka, *Psychol. Rev.* **108**, 483 (2001).
12. Materials and methods are available as supporting material on *Science* Online.
13. A mixed analysis of variance (ANOVA) conducted for acquisition trials revealed that participants exhibited greater CRs to outgroup than ingroup faces [$F(1, 71) = 4.03$, $P < 0.05$], an effect not qualified by participant race [$F(1, 71) = 0.85$, NS]. Likewise, a mixed ANOVA conducted for extinction trials revealed greater CRs to outgroup than ingroup faces [$F(1, 71) = 5.59$, $P < 0.05$], an effect not qualified by participant race [$F(1, 71) = 1.70$, NS]. In other words, participants acquired stronger CRs to outgroup relative to ingroup faces, a difference that remained pronounced during extinction.
14. D. L. Hamilton, R. K. Gifford, *J. Exp. Soc. Psychol.* **12**, 392 (1976).
15. C. O. Word, M. P. Zanna, J. Cooper, *J. Exp. Soc. Psychol.* **10**, 109 (1974).
16. Z. Kunda, K. C. Oleson, *J. Pers. Soc. Psychol.* **72**, 965 (1997).
17. H. Tajfel, J. C. Turner, in *The Social Psychology of Intergroup Relations*, W. Austin, S. Worchel, Eds. (Brooks/Cole, Monterey, CA, 1979), pp. 33–48.
18. T. F. Pettigrew, L. Tropp, *J. Pers. Soc. Psychol.*, in press.
19. M. E. P. Seligman, *Psychol. Rev.* **77**, 406 (1970).
20. A. Öhman, *Psychophysiology* **23**, 123 (1986).
21. K. Hugdahl, A. Öhman, *J. Exp. Psychol. Hum. Learn.* **3**, 608 (1977).
22. A. Öhman, J. Soares, *J. Abnorm. Psychol.* **102**, 121 (1993).
23. K. Hugdahl, A. C. Kärkner, *Behav. Res. Ther.* **15**, 345 (1981).
24. K. Hugdahl, B. H. Johnsen, *Behav. Res. Ther.* **27**, 269 (1989).
25. U. Dimberg, A. Öhman, *Motiv. Emot.* **20**, 149 (1996).
26. S. Molnar, *Human Variation: Races, Types, and Ethnic Groups* (Prentice Hall, Upper Saddle River, NJ, ed. 4, 1998).
27. W. D. Hamilton, *J. Theor. Biol.* **7**, 17 (1964).
28. J. H. Manson, R. W. Wrangham, *Curr. Anthropol.* **32**, 369 (1991).
29. A. G. Greenwald, D. E. McGhee, J. K. L. Schwartz, *J. Pers. Soc. Psychol.* **74**, 1464 (1998).
30. D. Katz, K. Braly, *J. Abnorm. Soc. Psychol.* **28**, 282 (1933).

31. We want to thank W. Brennan, D. Fareri, and N. Husain for helpful assistance; J. Eberhardt for providing the face stimuli; N. Shelton for providing the contact items; and A. G. Greenwald, J. R. Hackman, and R. L. Trivers for their helpful comments. This research was supported by the James S. McDonnell Foundation, a 21st Century award (E.A.P.), National Institute of Mental Health grants 1RO1MH57672 and 5R01MH068447 (M.R.B.), and an NSF graduate research fellowship (J.P.E.).

Supporting Online Material

www.sciencemag.org/cgi/content/full/309/5735/785/DC1
Materials and Methods
SOM Text
Fig. S1
Tables S1 to S5
References

13 April 2005; accepted 20 June 2005
10.1126/science.1113551

An Interneuronal Chemoreceptor Required for Olfactory Imprinting in *C. elegans*

Jean-Jacques Remy[1] and Oliver Hobert[2]

Animals alter their behavioral patterns in an experience-dependent manner. Olfactory imprinting is a process in which the exposure of animals to olfactory cues during specific and restricted time windows leaves a permanent memory ("olfactory imprint") that shapes the animal's behavior upon encountering the olfactory cues at later times. We found that *Caenorhabditis elegans* displays olfactory imprinting behavior that is mediated by a single pair of interneurons. To function in olfactory imprinting, this interneuron pair must express a G protein–coupled chemoreceptor family member encoded by the *sra-11* gene. Our study provides insights into the cellular and molecular basis of olfactory imprinting and reveals a function for a chemosensory receptor family member in interneurons.

Olfactory imprinting, which occurs in contexts as diverse as homing behavior in salmon and neonatal attachment in mammals, is a learned olfactory response whose defining features are that the olfactory memory is long-lasting and can only be acquired during a defined developmental time window or during a specific physiological state (*1*). These features distinguish it from other learned olfactory responses, such as olfactory adaptation, which can occur at many distinct developmental or physiological states and usually lasts for a limited amount of time. However, the cellular and molecular basis of olfactory imprinting is poorly understood.

[1]Laboratoire NMDA CNRS UMR 6156, Institut de Biologie du Développement (IBDM), 13288 Marseille Cedex 9, France. E-mail: remy@ibdm.univ-mrs.fr
[2]Howard Hughes Medical Institute, Department of Biochemistry and Molecular Biophysics, Center for Neurobiology and Behavior, Columbia University Medical Center, New York, NY 10032, USA. E-mail: or38@columbia.edu

To assess whether olfactory imprinting exists in *C. elegans*, we exposed worms to specific odorants over defined developmental time windows and then assayed odorant attraction in adult worms. Odorant attraction is classically assayed by quantifying the number of animals in a population capable of migrating up a gradient of a defined olfactory cue (*2*). To increase the sensitivity of the odorant attraction assays, we did not restrict ourselves to determining the number of animals that had accumulated at the source of an olfactory cue after a given time period but rather chose to closely monitor the kinetics of the migratory behavior of animals in an olfactory gradient. Specifically, we recorded the position of animals in the olfactory gradient at several distinct time points, thus allowing us to calculate a "migration index" (fig. S1) (*3*). This migration index is an indicator of the speed and efficiency with which animals can respond to olfactory cues.

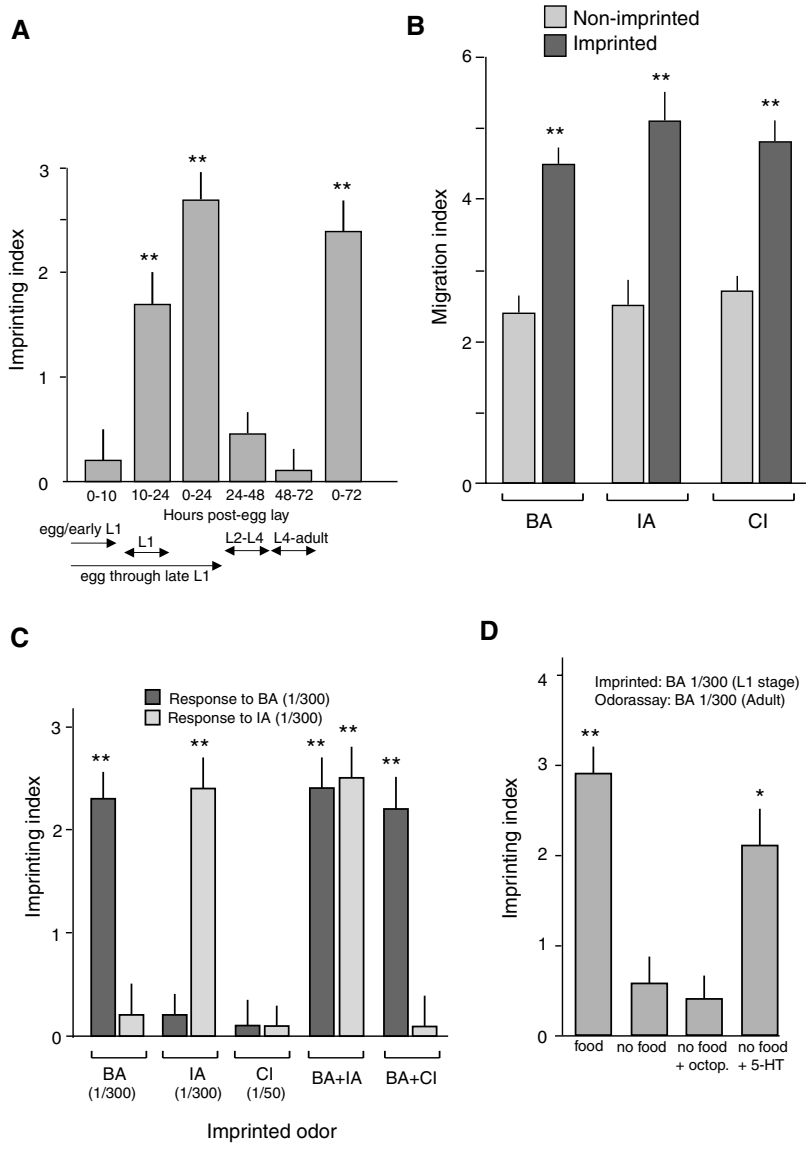


Fig. 1. *C. elegans* displays odor-specific olfactory imprinting. (**A**) Stage-specific olfactory imprinting with BA. Developing worms were exposed to BA diluted 1/300 in water at different developmental stages. Migration indices of 5-day-old animals were determined at an odorant concentration of 1/300. Each assay was done five times with 20 animals each. Interaction analysis with the two-way analysis of variance (ANOVA) test indicated a highly significant (**$P < 0.001$) impact of olfactory response depending on both olfactory imprinting and the development time course of imprinting. (**B**) Imprinting with BA, IA, and CI. The odorant concentrations for imprinting and for the attraction assay that followed were 1/300 for BA and IA and 1/50 for CI. The assays were done 10 times for BA, 7 times for CI, and 4 times for IA, with 20 worms tested in each assay. **$P \leq 0.005$, comparing imprinted and nonimprinted worms. (**C**) Olfactory imprinting is odorant-specific. Each assay was done three to five times with 20 animals each. Animals were imprinted at the L1 stage. The P value (**$P < 0.01$) refers to the significance of the comparison of the migration indices of naïve and imprinted worms (i.e., the imprinting index). See table S1 for assays at different odorant concentrations. (**D**) Odorant imprinting is food-dependent. BA (1/300) was presented during the imprinting period (L1 stage) in the presence or absence of food with or without 3 mM serotonin (n = 4, 20 animals each) or 3 mM octopamine (n = 3). **$P < 0.0001$, comparing food to no food; *$P <$ 0.05, comparing no food to no food + 5-HT (serotonin).

We found that preexposure of worms to the odorant benzaldehyde (BA) at a specific developmental stage significantly improved the ability of adult worms to migrate toward a BA source presented at moderately attractive concentrations (Fig. 1, A and B) (table S1). We express the impact of preexposure to an olfactory cue as an olfactory "imprinting index," which we define as the difference between the migration indices of preexposed ("imprinted") and non-preexposed ("naïve") animals. For example, the migration index in a BA gradient is 2.3 ± 0.23 for naïve worms and 4.5 ± 0.22 for imprinted worms (P = 0.0001; Fig. 1B), which translates into an imprinting index of 2.27 ± 0.24 (Fig. 1A).

Preexposure to BA must occur at a specific developmental window, coinciding with the first larval stage (Fig. 1A), therefore defining this learned olfactory behavior as olfactory imprinting. Odorant exposures before hatching or after the L1 stage produced no significant imprint.

Sensory inputs such as the presence of food profoundly affect egg-laying rate (*4*). We found that the presence of the olfactory cue BA also strongly affected egg-laying rate (fig. S2). Notably, the dose response values for the effect of BA on egg laying and odorant attraction (migration index) were strongly correlated. We therefore tested whether olfactory imprinting also affects egg-laying rate. Upon encountering BA in the adult stage, BA-imprinted adult wild-type worms laid up to twice as many eggs per hour as naïve worms (fig. S3). Olfactory imprinting therefore leads to a sensitization of two distinct motor outputs, locomotion, and egg laying. We note that the locomotory output of olfactory imprinting (i.e., the enhanced performance of imprinted animals in odorant attraction assays) also correlates with the reproductive state of the animal, because imprinted larval or prereproductive adult animals showed no enhanced response in odorant attraction assays (*5*).

The BA odorant is sensed by the AWC sensory neuron class (*2*). Two other AWC-sensed olfactory cues, isoamylalcohol (IA) and citronellol (CI), are similarly able to leave an olfactory imprint (Fig. 1B). In contrast, diacetyl, which is sensed by the AWA neuron class, is unable to leave an imprint (*5*). Because BA and IA are both sensed by the AWC sensory neurons, we tested whether imprints could be generated in an odorant-specific manner within one olfactory neuron class. Imprinting of animals with IA (or CI)

did not affect the response of animals to later encounters of BA (Fig. 1C) (table S1). Conversely, imprinting with BA did not affect their response to IA. Moreover, the simultaneous presence of odorants in addition to BA or IA did not affect the ability of BA or IA to leave an imprint. We conclude that olfactory imprints are not generated on the level of the whole receptor neuron, but are generated in an odorant-specific manner.

To investigate the potential physiological relevance of olfactory imprinting, we asked whether an olfactory imprint could be used as a memory device for favorable environmental conditions. One environmental condition that is known to affect a plethora of behavioral paradigms in *C. elegans* is the presence or absence of food [reviewed in (*6*)]. We found that the absence of food during the critical learning phase disrupted olfactory imprinting (Fig. 1D). Serotonin mimics the presence of food in various sensory paradigms (*6*), and the addition of exogenous serotonin into the food-free agar plates indeed restored olfactory imprinting (Fig. 1D). In contrast, octopamine, another monoamine present in *C. elegans*, did not compensate for food deprivation. We did not observe any significant enhancement of the responses when olfactory imprinting was carried out in the presence of both food and serotonin (*5*). If animals were starved and exposed to BA and serotonin at the adult stage, no improvement in the subsequent odorant attraction assay was observed, which indicates that the association of food and serotonin with an odorant occurs only during the critical olfactory imprinting period. Taken together, these results suggest that a potential function of olfactory imprinting is the memorization of favorable growth conditions.

The existence of olfactory imprinting in *C. elegans* afforded us the opportunity to start defining the as yet elusive cellular and molecular mechanisms of olfactory imprinting. In the olfactory imprinting phenomena associated with neonatal attachment in rats, it is thought that several different, though poorly defined, central brain areas play an important role (*7*). We focused our cellular analysis on the two bilaterally symmetric AIY interneurons, which receive synaptic inputs from several distinct classes of sensory neurons, including the BA- and IA-sensing AWC odorsensory neuron class (Fig. 2A). We genetically disabled the AIY interneurons by reducing the activity of the *ttx-3* homeobox gene, which controls the functional differentiation of AIY (*8*). Using olfactory cues at a concentration at which *ttx-3* mutants are still capable of responding to the cue, we found that preexposure of *ttx-3* mutants to odorants failed to leave an olfactory imprint (Fig. 3A). Because *ttx-3* is expressed in three other neuron classes besides AIY (*8*), we tested whether *ttx-3* indeed acts in AIY to affect olfactory imprinting. Driving expression of a *ttx-3* cDNA under control of a cis-regulatory element that is exclusively active in the AIY interneurons (*9*) rescued the olfactory imprinting defects of *ttx-3* mutant animals (Fig. 3A).

The *ttx-3* homeobox gene directly regulates the expression of scores of AIY-expressed genes, one of which is the *sra-11* gene (*8*). *sra-11* encodes an orphan, G protein–coupled seven-transmembrane receptor (7TMR) that belongs to a large family of putative chemoreceptor-encoding genes that were uncovered through genome sequence searches (*10*). Unlike other chemoreceptor family members, which are expressed in sensory neurons, *sra-11* is exclusively expressed in three interneuron classes, AIY, AIA, and AVB (*10*). Expression can be observed throughout all larval and adult stages (*8*). Two mutant alleles of *sra-11*, each likely null alleles that delete most if not all of the *sra-11* locus (Fig. 2B), were made available by the *C. elegans* Gene Knockout Consortium. Several assays that test the functionality of AIY, including reversal assays and thermokinesis assays, indicate that previously known aspects of AIY function are unaffected by the absence of *sra-11* (*3*, *11*). As assayed by odorant attraction assays as well as the odorant-induced egg-laying response, *sra-11* null mutants showed a normal response to several odorants tested, including BA (Fig. 3B) (fig. S3). However, the odorant response of *sra-11* null mutants failed to be positively imprinted by BA or IA (Fig. 3A) (fig. S4). Both *sra-11* null alleles showed similar olfactory imprinting defects. These olfactory imprinting defects could be measured on both motor output levels, the locomotory output (Fig. 3A) and the egg-laying output (Fig. 3C). Animals that lack another AIY-expressed 7TMR, the tyramine receptor *ser-2* (*12*), showed intact olfactory imprinting; hence, olfactory imprinting was not affected by the disruption of any AIY-expressed 7TMR.

Because *sra-11* is expressed in two other neuron classes besides AIY (*10*), we tested whether *sra-11* function is indeed required in AIY by generating transgenic *sra-11* null animals that express *sra-11* under control of an AIY-specific cis-regulatory element (*3*). As a control, we deleted the G protein–coupled C terminus of *sra-11* and generated animals expressing this construct under control of the same AIY-specific cis-regulatory element. Double-blind scoring of the transgenic lines revealed that only the wild-type construct was able to rescue the *sra-11* null mutant phenotype (Fig. 3, A and C).

Our analysis leads to three main conclusions: (i) *C. elegans* displays a learned olfactory response pattern that can be classified as olfactory imprinting. The imprint is associated with favorable growth conditions (food) and, in analogy to many other olfactory imprinting paradigms, is generated at an early juvenile stage. The imprinted odorant increases the attraction of a mature animal to this odorant and stimulates egg laying, so as to allow the progeny of the animal to exploit the memory of these favorable environmental conditions. (ii) Olfactory imprinting requires a single interneuron pair that is postsynaptic

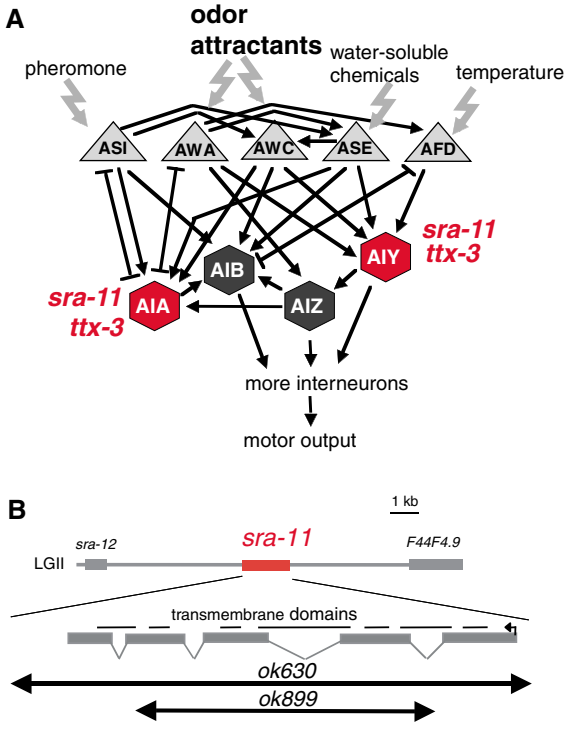


Fig. 2. Cells and genes tested for an effect on olfactory imprinting. (**A**) Schematic representation of the synaptic connectivity of the AIY interneuron class (*14*) and the sites of *ttx-3* and *sra-11* expression within this circuit. (**B**) *sra-11* locus and structure of mutant *sra-11* alleles.

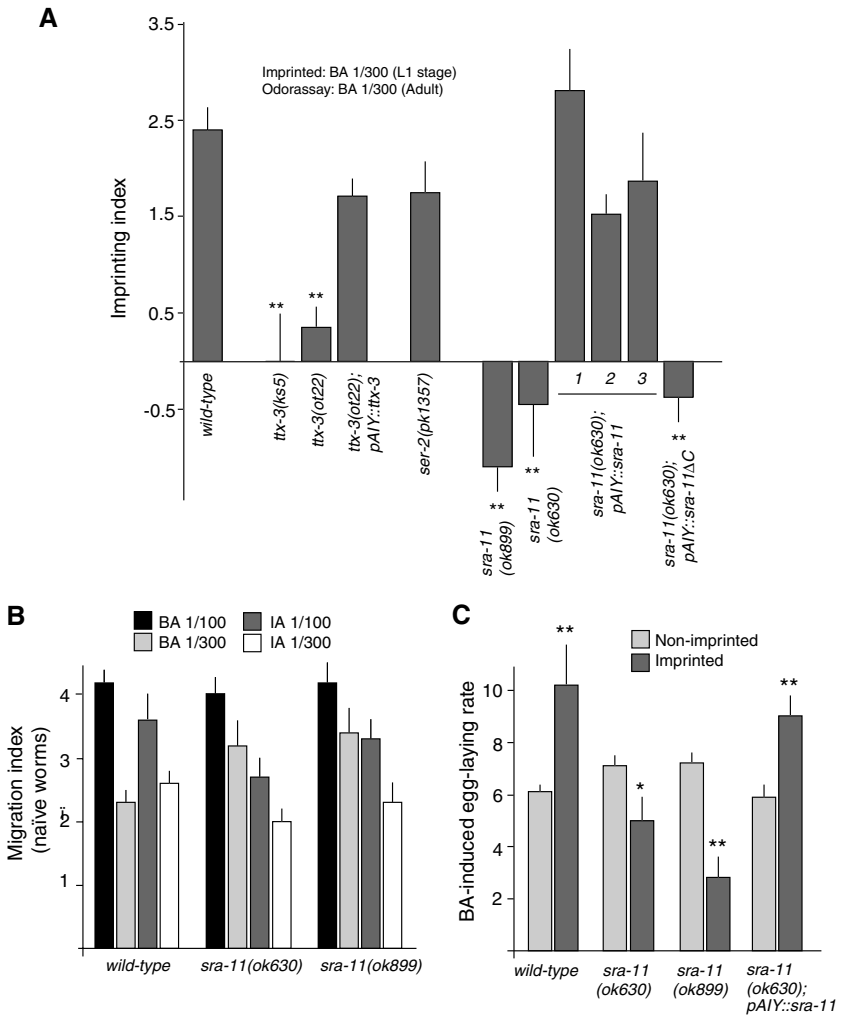


Fig. 3. AIY-expressed *sra-11* is required for olfactory imprinting. (**A**) *ttx-3* and *sra-11* mutant animals show imprinting defects for BA as measured in an odorant attraction assay. Three independent rescued lines that express *sra-11* exclusively in AIY ("*pAIY::sra-11*") and one control transgenic line expressing a truncated *sra-11* gene in AIY ("*pAIY::sra-11ΔC*") are shown. See table S2 for complete data set. $^{**}P < 0.001$ for comparison of wild-type to both *ttx-3* and both *sra-11* alleles and to the control line for rescue and for the comparison of both *ttx-3* mutants to the rescued line and the *sra-11* mutants to the rescued lines. (**B**) *sra-11(ok630)* null mutants show a normal response to different concentrations of BA and IA. Each assay was done in triplicate with 20 animals each. (**C**) *sra-11* mutants also show imprinting defects in the egg-laying imprinting assay. One representative rescued line is shown. Values are the means of a total of 80 worms (two independent experiments with 10 worms each on four plates per condition). $^{**}P < 0.01$, $^{*}P < 0.05$, comparing imprinted and nonimprinted animals under each condition. Note that *sra-11* mutants appear to display a negative olfactory imprint, given that imprinted animals are less attracted to BA [as shown in (A)] and lay fewer eggs relative to naïve worms.

to olfactory neurons. (iii) The SRA-11 protein, a member of a large chemosensory receptor family, is specifically required for olfactory imprinting. Surprisingly, SRA-11 does not function in sensory neurons but in interneurons downstream of the sensory neuron class to control olfactory imprinting. Because the olfactory imprinting process shows odorant selectivity, and because olfactory imprinting to at least two distinct odorants is disrupted in *sra-11* mutants, we infer that *sra-11* is required for a generic rather than odorant-specific aspects of olfactory imprinting. The SRA-11 protein could be a generic subunit of a receptor complex that is activated by an AWC-released ligand upon imprinting by distinct odorants, leaving permanent marks in the AIY interneuron; upon a later encounter of the same odorant by the AWC neuron class, these marks may facilitate signaling through the AIY interneuron. In analogy to glomerular targeting mediated by vertebrate olfactory receptors (*13*), it is also conceivable that SRA-11 may have a role in determining fine aspects of AWC-AIY connectivity that may be modulated upon olfactory imprinting. Elucidating the nature of the ligand of the SRA-11 protein will provide further insights into the process of olfactory imprinting.

References and Notes

1. R. Hudson, *Curr. Opin. Neurobiol.* **3**, 548 (1993).
2. C. I. Bargmann, E. Hartwieg, H. R. Horvitz, *Cell* **74**, 515 (1993).
3. See supporting data on *Science* Online.
4. C. Trent, thesis, Massachusetts Institute of Technology (1982).
5. J.-J. Remy, O. Hobert, data not shown.
6. O. Hobert, *J. Neurobiol.* **54**, 203 (2003).
7. R. M. Sullivan, *Ann. N.Y. Acad. Sci.* **1008**, 122 (2003).
8. Z. Altun-Gultekin *et al.*, *Development* **128**, 1951 (2001).
9. E. L. Tsalik, O. Hobert, *J. Neurobiol.* **56**, 178 (2003).
10. E. R. Troemel, J. H. Chou, N. D. Dwyer, H. A. Colbert, C. I. Bargmann, *Cell* **83**, 207 (1995).
11. E. L. Tsalik, thesis, Columbia University Medical Center (2003).
12. E. L. Tsalik *et al.*, *Dev. Biol.* **263**, 81 (2003).
13. F. Wang, A. Nemes, M. Mendelsohn, R. Axel, *Cell* **93**, 47 (1998).
14. J. G. White, E. Southgate, J. N. Thomson, S. Brenner, *Philos. Trans. R. Soc. London Ser. B* **314**, 1 (1986).

15. We thank the *C. elegans* Gene Knockout Consortium in Oklahoma for providing the *sra-11* knockout alleles; M. A. Felix (Institut Jacques Monod) and all members of her group; S. Lockery for help with statistics; I. Greenwald, P. Sengupta, and members of the Lockery and Hobert lab for comments on the manuscript; J. Zhu for generating the *sra-11* constructs; and Q. Chen for expert DNA injection assistance. Supported by the Neurobiologie de l'Olfaction et de la Prise Alimentaire laboratory, the Physiologie Animale et Systèmes d'Elevage (PHASE) department of Institut National de la Recherche Agronomique (J.-J.R.), and NIH grant NS039996-05 (O.H.).

Supporting Online Material

www.sciencemag.org/cgi/content/full/309/5735/787/DC1
Materials and Methods
Figs. S1 to S4
Tables S1 and S2

28 April 2005; accepted 17 June 2005
10.1126/science.1114209

Reversible Protein Detection Kit

The ZiP Reversible Protein Detection Kit can destain in less than 5 min and provide results in 15 min. The kit allows for fast and reversible visualization of proteins in a polyacrylamide gel suitable for use with downstream analysis such as sequencing, mass spectrometry, and protein immunoblotting. It allows obtaining of sequence data from proteins not detected on transfer membranes and offers an established method of detection when structural analysis is required.

Amresco For information 800-448-4442 www.amresco-inc.com

Spectrometer for Raman Analysis

The QE65000 Spectrometer is a high-sensitivity modular spectrometer for Raman analysis and other low-light applications such as fluorescence, DNA sequencing, thin film reflectivity, and astronomy. The QE65000 can achieve up to 90% quantum efficiency with high signal-to-noise ratio and rapid signal processing speed. A user can optimize the spectrometer's range and resolution by selecting from 15 gratings and seven entrance slit options. The QE65000 features an SM 905 connector and accepts light transmitted through an optical fiber, which enables users to make Raman measurements using their own laser and probe configuration. The manufacturer offers a series of fiber optic Raman probes, each providing complete optical filtering of the Rayleigh line and high signal collection in a compact, rugged design. Probe models are available for laboratory, industrial, and environmental applications and are available with 514 nm, 532 nm, 633 nm, 670 nm, 783 nm, 785 nm, and 830 nm bandpass filters.

Ocean Optics For information 727-733-2447 www.oceanoptics.com

Biomarker Discovery and Screening Platform

The Bioexpression Biomarker discovery and screening platform is designed to speed the discovery of biomarkers through unique biomarker sample enrichment technology and highly accurate mass pattern recognition. The platform introduces Proteomic Signature Technology, which delivers high throughput and high mass accuracy and stability, resulting in comprehensive and high-confidence proteomic signatures.

Perkin Elmer For information 617-587-2814 www.perkinelmer.com

DNA Concentrator

The miVac DNA concentrator is designed to prepare DNA samples efficiently and safely. It offers digital control of concentrator temperature and short concentration times, reducing the risk of heat damage to samples. Unlike centrifugal filters, the miVac concentrates effortlessly and returns 100% of the samples every time, with no consumable costs. The design features no motor and therefore runs very quietly.

Barnstead Genevac For information +44-1473-240000 www.mivac.co.uk

Small Interfering RNA

A portfolio of small interfering RNA (siRNA), used for analyzing the function of genes and for silencing gene activity, has been extended with the addition of multiple scales and siRNA in either the classic 21mer or the new 27mer format. This new format has recently attracted much interest as it is assumed to provide higher efficiency. The line now offers three different scales (0.05 μmol, 0.2 μmol, and 1.0 μmol), with improved yields on the already existing 0.2 μmol scale. There is also a new advanced siRNA online design tool. The manufacturer offers licensed high-quality custom siRNA produced with proprietary technology.

MWG Biotech For information +49 8092 8289 929 www.mwg-biotech.com

Fast PCR System

The Applied Biosystems 9800 Fast PCR System delivers polymerase chain reaction (PCR) results in a standard 96-well plate in approximately 25 min. Consisting of the 9800 Fast Thermal Cycler, the GeneAmp Fast PCR Master Mix, and the 96-well Fast Thermal Cycling Plate, the system is designed to enable researchers to perform routine PCR procedures, such as cloning and preparing DNA for sequencing, faster, more easily, and more conveniently. Scientists often share PCR instruments, and this new system can alleviate some of the burden of instrument-sharing by reducing cycling time.

Applied Biosystems For information 800-327-3002 www.appliedbiosystems.com

Electron Transfer Dissociation

An Electron Transfer Dissociation (ETD) module for a top-of-the-line high-capacity ion trap mass spectrometer (MS), the HCTultra, is a unique new peptide and protein fragmentation technique that preserves functionally important post-translational modifications (PTMs), such as phosphorylation or glycosylation. With ETD in a high-capacity ion trap, protein scientists can sequence peptides easily, and in addition they can simultaneously identify both the type and location of various PTMs. ETD MS/MS spectra of peptides can be collected on-the-fly during liquid chromatography/MS/MS runs using the HCTultra. On the HCTultra, the new ETD implementation often provides complete amino acid series down to immonium ion masses, without the low-mass cut-off traditionally encountered in ion trap MS/MS.

Bruker Daltonics For information 978-667-9580 www.bruker-biosciences.com

DNA Polymerase

The Phusion DNA polymerase generates templates with an accuracy and speed previously unattainable with a single enzyme. The unique structure and characteristics set a new standard for polymerase chain reaction performance. Its error rate is 50-fold lower than that of Thermus aquaticus and six-fold lower that that of Pyrococcus furiosus, according to the manufacturer. It possesses processivity 10-fold greater than Pyrococcus furiosus and twice that of Thermus aquaticus.

Finnzymes Oy For information +358 9 584 121 www.finnzymes.fi

Newly offered instrumentation, apparatus, and laboratory materials of interest to researchers in all disciplines in academic, industrial, and government organizations are featured in this space. Emphasis is given to purpose, chief characteristics, and availability of products and materials. Endorsement by *Science* or AAAS of any products or materials mentioned is not implied. Additional information may be obtained from the manufacturer or supplier by visiting www.science.labvelocity.com on the Web, where you can request that the information be sent to you by e-mail, fax, mail, or telephone.

TaKaRa

Coming soon!

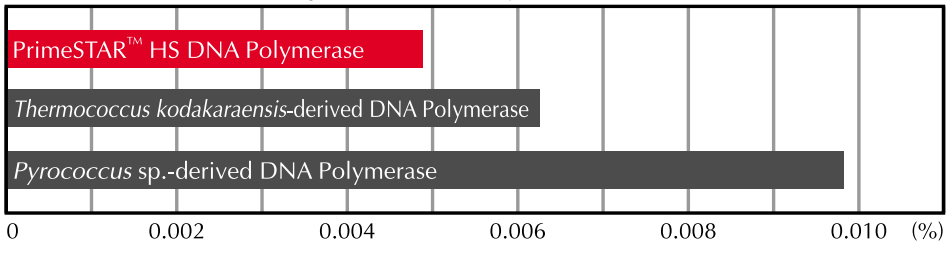
Hungry for a new high fidelity PCR polymerase?
Try PrimeSTAR™ HS DNA Polymerase...extreme fidelity.

PrimeSTAR™ HS DNA Polymerase is a novel PCR enzyme that provides maximum fidelity as well as extended product length. Targeted for PCR products intended to be cloned, it offers extremely high accuracy together with high amplification efficiency.

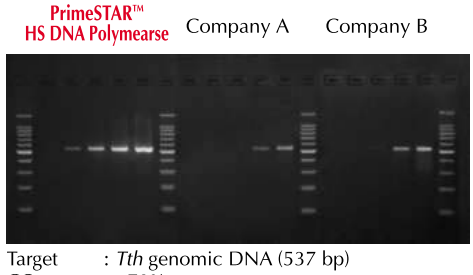
Get a taste of easy, successful, high fidelity PCR with PrimeSTAR™ HS DNA Polymerase!

Extremely Low Error Rate

Sequencing results showed only 12/250,000 mismatched bases in DNA fragments amplified by PrimeSTAR™ HS, demonstrating incredible accuracy!



Excellent Amplification Efficiency



Target : *Tth* genomic DNA (537 bp)
GC-content : ~70%

TaKaRa PCR Related Products are sold under licensing arrangements with F. Hoffmann-La Roche Ltd, Roche Molecular Systems, Inc. and Applied Biosystems.

TAKARA BIO INC.
The Biotechnology Company™

Otsu, Shiga, Japan
Phone: +81 77 543 7247 Fax: +81 77 543 9254

USA: **Takara Mirus Bio Inc.** Toll Free Phone: 888 251 6618 Fax: +1 608 441 2845
Europe: **Takara Bio Europe S.A.S.** Phone: +33 1 41 47 23 70 Fax: +33 1 41 47 23 71
Korea: **Takara Korea Biomedical Inc.** Phone: +82 31 739 3300 Fax: +82 31 739 3311
China: **Takara Biotechnology (Dalian) Co., Ltd.** Phone: +86 411 8764 1681 Fax: +86 411 8761 9946

For more information and a list of Takara distributors worldwide, please visit our website today!

www.takara-bio.com

» advances in:

Genomics

In this issue:

- Production methods for siRNAs
- Commercially available siRNAs
- Isolating and purifying RNA
- Transfection methods for siRNA
- Therapeutic applications of gene silencing

Silent Revolution RNA interference, one of the most rapidly developing technologies in life science, has potential applications beyond the research laboratory. Investigators have begun to study its use in medical therapy.

BY PETER GWYNNE AND GARY HEEBNER

Every few years, a revolutionary technology emerges that fundamentally changes life scientists' ability to undertake research and development and to apply their findings. The emergence of gene silencing represents a recent example of the phenomenon. Otherwise known as RNA interference (RNAi) or short interfering RNA (siRNA), this is a biological process that suppresses the expression of active genes in living cells. During the past four years it has taken the world of life science by storm. "To say that siRNA is growing rapidly is an understatement," says Spyro Mousses, head of cancer drug development at the **Translational Genomics Research Institute** (TGen). "The siRNA field is growing exponentially," agrees Becky Mullinax, senior staff scientist at **Stratagene**. "Scientists have needed this tool for a long time."

The technology has a variety of real and potential applications. "We're seeing traction in a lot of areas," says Bill Marshall, executive vice president for research and operations manager at **Dharmacon**. "For academic, pharmaceutical, and biotech researchers working at the cell culture level, this has become the dominant gene regulation technology," says Barry Polisky, senior vice president for research and chief scientific officer at **Sirna Therapeutics**. It also has high promise beyond the lab. "It is a real breakthrough in molecular biology – a new and very exciting approach for gene therapy," explains Franck Montagne, chief scientific officer of **Elchrom Scientific**.

Inclusion of companies in this article does not indicate endorsement by either AAAS or Science*, nor is it meant to imply that their products or services are superior to those of other companies.*

This is the third of four special supplements this year on Advances in Genomics. The first two appeared in the 11 February and 10 June issues of Science*; the final one will appear in the 14 October issue.*

Asking Questions

Originally observed in the 1980s in flowers, followed quickly by worms and fruit flies, siRNA began to catch the attention of life science researchers in 2001, with publication of the first report of its existence in mammalian systems. Since then, reports Walter Tian, business director for gene silencing at **Qiagen**, "More and more people have been asking questions about RNA interference and wanting to know how they can get started."

RNA interference isn't the only method that scientists can use to silence genes. "People have had experience with antisense and ribozymes," Polisky explains. "But siRNA has demonstrated itself to be the most useful of this species of tools for a number of reasons. The most important is that it engages an endogenous mechanism that exists in cells."

The process begins when scientists introduce double-stranded RNA (dsRNA) into a cell. Once inside the cell, an enzyme known as the dicer cleaves long stretches of the dsRNA into short interfering RNAs or microRNAs (miRNAs) 21 to 28 nucleotides in length. Those short RNAs then associate with proteins to form an RNA-induced silencing complex (RISC) that binds to complementary messenger MORE ›››

» advances in: Genomics

RNAs and stimulates their degradation by exonucleases. That causes the corresponding genes to be silenced.

Scientists have identified RNA interference in virtually every cell type outside bacteria and yeasts. In its natural state it facilitates antiviral defense, gene regulation, and genomic rearrangements. But scientists value the capacity that siRNA gives them to calibrate their control of genes. "It's not a knockout technology but a knockdown one," Polisky explains. "A researcher can knock down a gene of interest by introducing a short strand of DNA into cells." Beyond that, Mousses adds, "The biggest advantage of siRNA is the ability to inhibit nontractable targets – those that can't be inhibited by other biologicals or those that are not accessible because there's no way of getting selectivity at the protein level. With siRNAs, it's theoretically possible to inhibit anything in the proteome; they're in a class of their own."

Methods of Production

Methods of producing siRNAs include chemical synthesis, in vitro transcription, and digestion of long dsRNA by a dicer enzyme in the cell. "The synthetic approach has the advantage of controlling both purity and the final sequence. The disadvantage is that the obtained synthetic DNA must be successfully transfected into the cell," says Anatoli Tassis, manager of business development for oligo purification at Elchrom Scientific. "On the other hand, if RNA is intracellularly synthesized, this leads to an ongoing inhibition."

Each method has unique features and benefits appropriate to different types of investigation. Chemical synthesis, for example, is well suited for studies that require a large amount of defined siRNA, while in vitro transcription methods are ideal for screening siRNA sequences or short-term studies. Digestion of long dsRNA produces a heterogeneous population of siRNAs but does not require the design and testing of specific siRNA sequences beforehand.

Cambridge BioScience, Stratagene, and **Upstate**, among other vendors, offer kits that permit scientists to produce and assay their own siRNAs. "We were one of the first companies to offer the recombinant dicer," says Stratagene's Mullinax. "We also have transfection reagents and two GeneEraser suppression test kits that detect the effectiveness of siRNA reagents by suppression of a reporter gene." The company also plans to offer tools for micro RNAs.

Researchers can buy siRNAs for many different genes from such vendors as **Ambion**, Dharmacon, and Qiagen. These suppliers offer tested off-the-shelf siRNAs and also synthesize custom siRNAs for researchers. Ready-to-use siRNAs offer the benefit of being validated for gene silencing, which can save both the time and effort necessary to develop them from scratch. "If there's a synthetic siRNA available that has been shown to be effective, I would buy it," Mullinax notes.

All About Microarrays

In fall, the 12th annual Chips to Hits conference will give life scientists the opportunity to discover everything they want to know about microarrays and microtechnology. The event, organized by **IBC Life Sciences** and held at the Boston Convention and Exhibition Center from September 12 to September 15, will feature four keynote speakers, presentations by more than 70 industry leaders, launches of new products, more than 100 exhibition booths, and 75 scientific posters.

The conference will cover the entire microarray spectrum from emerging technologies to soon-to-be-commercialized products and validated case studies of current applications. Its main tracks will include U.S. Food and Drug Administration and government perspectives, scientific and commercial strategies, validated applications, and sessions on molecular diagnostic tools and technology and biomarkers for molecular diagnostics.

You can find more information about the event at the website

» http://www.chipstohits.com

Qiagen typifies that type of supplier. "We offer three major product types: predesigned siRNA for genomewide content, functionally validated siRNA, and custom siRNA design and synthesis," Tian says. "We design them all with our state-of-the-art HiPerformance siRNA design algorithm that uses a neural network approach to design the most potent and specific siRNA." The firm's recent offerings include HP GenomeWide siRNA for knockdown of all human, mouse, and rat genes using predesigned siRNAs.

Isolation, Purification, and Transfection

Traditional procedures for isolating RNA focus on recovering larger RNAs, such as transfer, ribosomal, and messenger RNA. Isolating siRNA, however, demands approaches that work well with smaller RNAs. And to avoid contamination, users must ensure that all solutions and materials stay free of RNase. "Purity is crucial in the use of RNA for gene silencing," Elchrom's Montagne says. Vendors such as **Bio-Rad Laboratories** and **GE Healthcare** offer a wide range of electrophoresis systems that can quickly and effectively separate different species of RNA.

Elchrom has developed a submerged gel electrophoresis system for high throughput RNA analysis and purification. "In combination with Elchrom's unique hydrogels, this system provides siRNA with very high purity," Montagne says. In addition, the company is currently developing a new generation of hydrogels for purifying RNA to a clinical grade.

Having purified their siRNA, researchers must insert it into living cells. "The challenge," Sirna's Polisky says, "is to deliver the material from the outside into the cytoplasm." Scientists can choose between physical and chemical means. "Chemical methods tend to use cationic liposomes or polymer carriers to uptake siRNA. They have probably the broadest application," Dharmacon's Marshall says. "The main physical method is electroporation. It suffers in not being a high throughput method and in causing distress to cells. We use it only where we can't find different sources of action in terms of chemical methods."

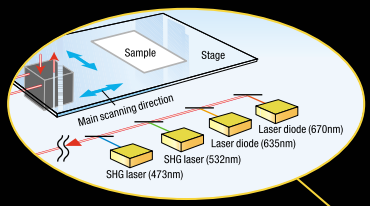
Dharmacon has designed its DharmaFECT transfection reagents specifically to deliver siRNA into cells. "Traditional lipids were MORE ›››

FUJIFILM
I&I – Imaging & Information

Life Science

Introducing Fujifilm's new FLA-5100 Advanced Imaging System.

THE FUTURE OF SCIENCE IMAGING IS IN THE DETAILS.



Four Internal Lasers Available

Wavelength 473, 532, 635, 670nm.
Wide range of applications.

FLUOR Stage

Watertight, washable.

IP Stage

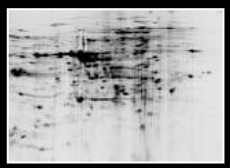
For phosphor imaging plate, scanning area 40 X 46cm.

Filter Module

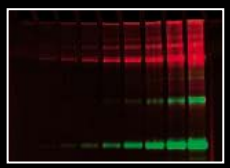
Easy to access, changeable by users.

APPLICATION VERSATILITY

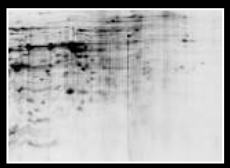
The new FLA-5100 imaging system allows imaging of fluorescent, radioisotopic, stained and chemiluminescent samples. This system is especially suitable for fluorescent (SYPRO® Ruby) detection or digitizing (CBB, silver stain) protein detection in the proteomics field.



Fluorescent SYPRO Ruby stain of 2D gel electrophoresis of Sake yeast.



2-Laser-2-Image overlap image with pseudocolor.



Fluorescent CBB stain detection by 670nm optional laser.

FEATURES

- Scanning area 46 X 40cm
- 2 Laser, 2 Image detection by dual PMT
- Fluorescent / Radioisotopic / Digitize / Chemiluminescent
- FLUOR / IP / MULTI Stage
- Pixel size – user choice of 10, 25, 50, 100, 200 microns
- Highly sensitive ^{3}H detection by BAS-TR IP

Japan Tel: +81-3-3406-2201
Fax: +81-3-3406-2158
http://home.fujifilm.com/products/science/index.html
Email: sginfo@tokyo.fujifilm.co.jp

Europe Tel: +49-211-5089-174
Fax: +49-211-5089-139
http://www.fujifilm.de
E-mail: mkaling@fujifilmeurope.de

U.S.A. Tel: +1-203-324-2000 ext. 6112
(1-800-431-1850 ext. 6112 in the U.S.A.) Fax: +1-203-351-4713
http://www.fujimed.com
E-mail: SSG@fujimed.com

Institutional Site License Available

Q What can *Science* STKE give me?

A The definitive resource on cellular regulation

STKE – Signal Transduction Knowledge Environment offers:

- A weekly electronic journal
- Information management tools
- A lab manual to help you organize your research
- An interactive database of signaling pathways

STKE gives you essential tools to power your understanding of cell signaling. It is also a vibrant virtual community, where researchers from around the world come together to exchange information and ideas. For more information go to www.stke.org

To sign up today, visit promo.aaas.org/stkeas

Sitewide access is available for institutions.
To find out more e-mail stkelicense@aaas.org

Science stke AAAS

» advances in: Genomics

designed as sledgehammers; we've created the ball peen hammer of the siRNA world," Marshall points out. "Our transfection reagents penetrate softly rather than slamming into the cells. They give researchers a lot more flexibility, and hence the ability to do more assays."

Several other companies offer chemical or physical supplies for transfection. Qiagen, for example, has recently introduced its HiPerFect transfection reagent for efficient delivery of submolar concentrations of siRNA into difficult to transfect cells. The list of vendors also includes **Amaxa**, **Gene Therapy Systems**, and **Mirus**.

Uses in the Lab and the Clinic

Short interfering RNA technology has found several applications in the research laboratory. "Most people are using siRNA to block expression of a gene," Elchrom's Tassis says. "In this way, scientists can analyze the function of genes of interest or see if other genes are taking over their functions." Validating gene targets has also emerged as a significant application. "You can inhibit a gene and infer from some relevant assay that the gene helps to correct some specific disease," Marshall explains. "We're also seeing indications of its use for target identification and validation in one step. You develop an assay screen by gene and find novel genes that carry out some function. And we're starting to see more applications in pathway assembly."

Tian of Qiagen sees the emergence of two other trends in the use of siRNA. "First is high throughput screening using genomewide siRNA libraries such as our Druggable Genome siRNA Set," he says. "The second trend is to apply RNAi technology to therapeutic uses, from lab animals to human clinical trials."

Therapeutic methods based on RNA interference have potentially significant advantages over traditional approaches to treating diseases. For example, drugs based on siRNA can target virtually any protein without the class restrictions seen in some small-molecule drugs. They can probably simplify the process of drug discovery, as they may not require the extensive lead optimization necessary with small-molecule drugs. And because they are designed to inhibit expression of only those genes associated with specific diseases, RNA-based drugs may reduce or avoid completely some of the side effects associated with traditional drugs. Several biotechnology companies, including **Acuity Pharmaceuticals** and **Alnylam Pharmaceuticals** are betting on siRNA for their therapeutic programs.

Targeting Cancers and Other Conditions

TGen uses siRNAs to discover weaknesses in cancer cell lines. "We're doing phenotype screening of the druggable genes from Qiagen," Mousses says. "We knock down thousands of genes in parallel and ask which ones are essential for the growth and survival of a cancer cell. We're also using high throughput siRNA to modify drug response."

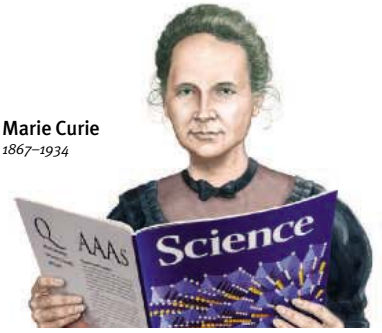
Early last year, Sirna began a joint collaboration to investigate its proprietary siRNAs against specific oncology targets provided by **Eli Lilly and Company**. Sirna is also exploring possible methods of delivering siRNA drugs. "We have both a modified siRNA and a delivery system that gives efficient systemic delivery in the liver," Polisky says. "We're also in a phase 1 clinical trial in which we inject into the eye a single species of siRNA directed against a single receptor that we believe is an important player in the angiogenesis associated with macular degeneration."

The relative simplicity of using siRNA for gene silencing has many researchers excited and eager to use this new research tool. Suppliers have developed tested siRNAs, siRNA libraries, kits, and reagents for research use. And pharmaceutical companies are exploring therapeutic uses of the tool. The rapid development of the technology so far indicates a bright future for the approach.

Peter Gwynne (pgwynne767@aol.com) is a freelance science writer based on Cape Cod, Massachusetts, U.S.A. Gary Heebner (gheebner@cell-associates.com) is a marketing consultant with Cell Associates in St. Louis, Missouri, U.S.A.

ScienceCareers.org

Classified Advertising



Marie Curie
1867–1934

For full advertising details, go to **www.sciencecareers.org** and click on **How to Advertise,** or call one of our representatives.

United States & Canada

E-mail: advertise@sciencecareers.org
Fax: 202-289-6742

JILL DOWNING
(CT, DE, DC, FL, GA, MD, ME, MA, NH, NJ, NY, NC, PA, RI, SC, VT, VA)
Phone: 631-580-2445

KRISTINE VON ZEDLITZ
(AK, AZ, CA, CO, HI, ID, IA, KS, MT, NE, NV, NM, ND, OR, SD, TX, UT, WA, WY)
Phone: 415-956-2531

KATHLEEN CLARK
Employment: AR, IL, LA, MN, MO, OK, WI, Canada; Graduate Programs; Meetings & Announcements (U.S., Canada, Caribbean, Central and South America)
Phone: 510-271-8349

EMNET TESFAYE
(Display Ads: AL, IN, KY, MI, MS, OH, TN, WV; Line Ads)
Phone: 202-326-6740

BETH DWYER
(Internet Sales Manager)
Phone: 202-326-6534

Europe & International

E-mail: ads@science-int.co.uk
Fax: +44 (0) 1223-326-532

TRACY HOLMES
Phone: +44 (0) 1223-326-525

HELEN MORONEY
Phone: +44 (0) 1223-326-528

CHRISTINA HARRISON
Phone: +44 (0) 1223-326-510

SVITLANA BARNES
Phone: +44 (0) 1223-326-527

JASON HANNAFORD
Phone: +81 (0) 52-789-1860

To subscribe to *Science*:
In U.S./Canada call 202-326-6417 or 1-800-731-4939
In the rest of the world call +44 (0) 1223-326-515

Science makes every effort to screen its ads for offensive and/or discriminatory language in accordance with U.S. and non-U.S. law. Since we are an international journal, you may see ads from non-U.S. countries that request applications from specific demographic groups. Since U.S. law does not apply to other countries we try to accommodate recruiting practices of other countries. However, we encourage our readers to alert us to any ads that they feel are discriminatory or offensive.

ScienceCareers.org
We know science AAAS

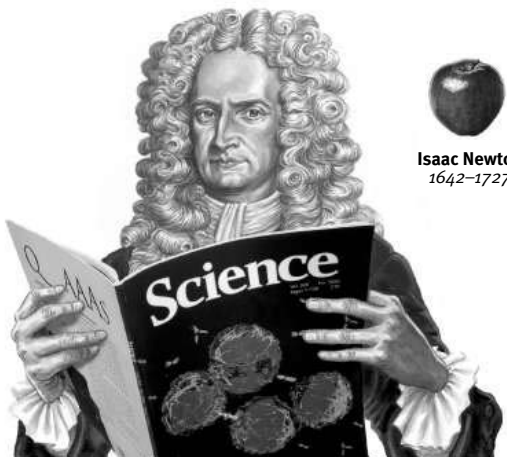
Largest bonus distribution of the year

Careers and Graduate Programs for

B.S. & M.S. Scientists

A *Science* Advertising Supplement

Issue date 26 August 2005
Reserve ad space by 9 August 2005



Isaac Newton
1642–1727

Largest Bonus Distribution of the Year!

Over 8,500 issues distributed to the top U.S. Biology and Chemistry programs. Total global weekly readership of over 710,000* that includes over 18,000 B.S. and M.S. level *Science* subscribers.

ScienceCareers.org postings available—7 websites for the price of one! If you post your job online, it is also searchable on these websites: Stanford University School of Medicine, National Postdoctoral Association (NPA), Biocompare, *Science*'s Signal Transduction Knowledge Environment (STKE), *Science*'s Aging Knowledge Environment (SAGE), and *Science*'s Next Wave.

1 *Science* June 2004 circulation of 129,590 as applied to 14 January 2000 Harvey Research Readership Survey and 2002 Harvey Research Cumulative Report, publisher's own data.

For more information, contact Daryl Anderson:
202-326-6543 – advertise@sciencecareers.org

ScienceCareers.org
We know science AAAS

Careers in Drug Discovery

Handling the Handoff

MOVING MOLECULES WITH THERAPEUTIC PROMISE FROM DISCOVERY TO DEVELOPMENT DEMANDS CLOSE COLLABORATION AMONG SCIENTISTS TRAINED IN A VARIETY OF DISCIPLINES. INDIVIDUALS INTERESTED IN THIS TRANSLATIONAL WORK MUST DEMONSTRATE FLEXIBILITY, COLLEGIALITY, AND COMMUNICATION SKILLS AS WELL AS EXCELLENT SCIENTIFIC BACKGROUNDS. BY PETER GWYNNE

Once upon a time, corporate drug discovery and drug development teams resembled Rudyard Kipling's East and West: Ne'er the twain would meet. Once they had isolated a molecule with therapeutic promise, discovery scientists would toss it over the wall to the development group and move on to other projects.

No more. "There's no throwing over the fence," says Lex van der Ploeg, vice president for basic research at Merck Research Laboratories in Boston. "If you do that, you lose critical information on the molecule that you need as you move forward into development."

So today East not only meets West in modern drug development: The two actively collaborate. Many pharmaceutical and biopharmaceutical firms regard teamwork as the essence of the early stages of drug development. Members of drug discovery teams collaborate with development groups by gaining some clinical understanding of the diseases they plan to combat and by being prepared to accompany the molecules they create into the development process and beyond. And development scientists frequently participate in the discovery process almost from the start. "Creating superior medicines requires both drug discovery informed by clinical knowledge *and* scientifically advantaged development strategies," says Robert Stein, president of Roche Palo Alto. CONTINUED »

» **Abbott**
http://www.abbott.com

» **AstraZeneca R&D**
http://www.astrazeneca.com

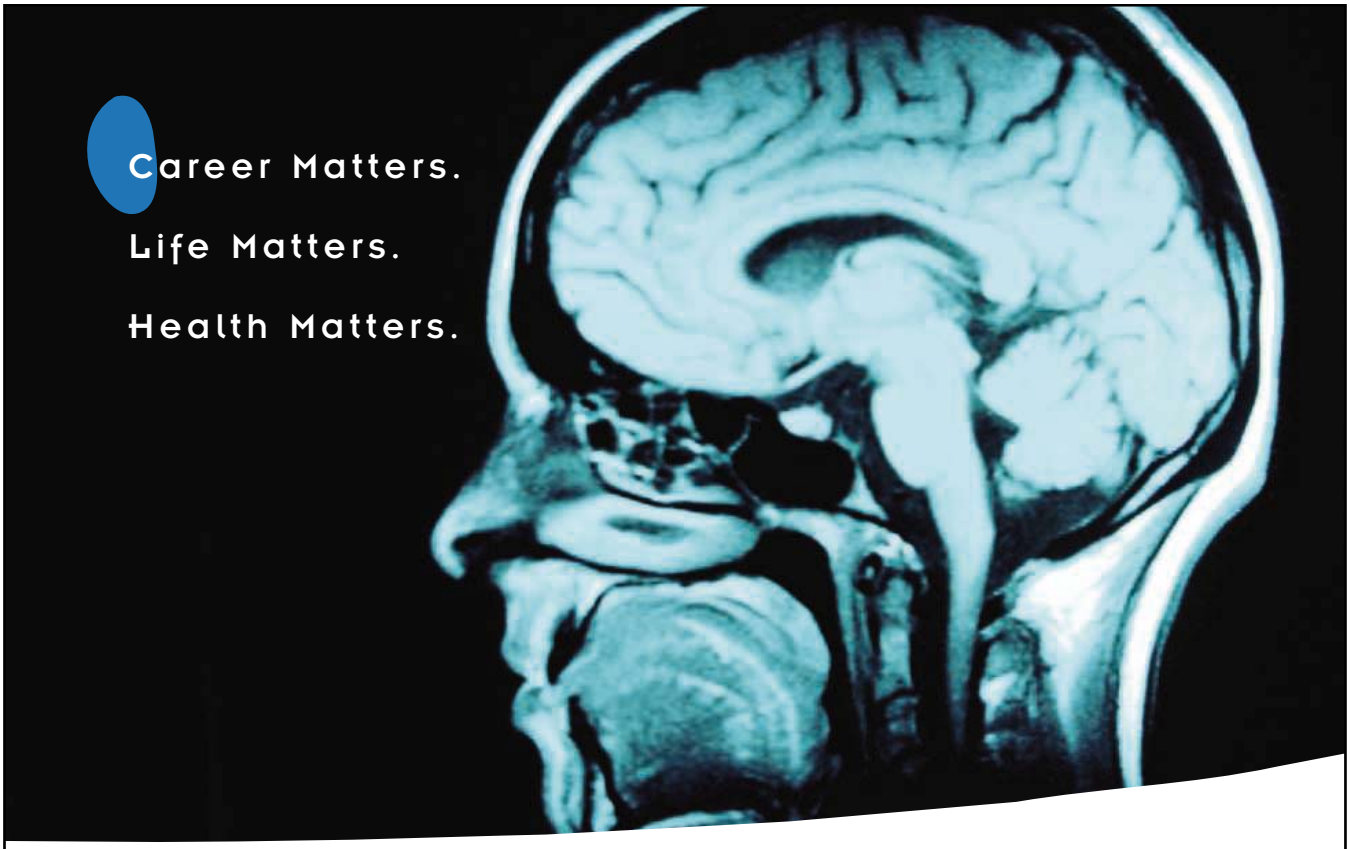
» **Biogen Idec**
http://www.biogenidec.com

» **IPS Pharma**
http://www.ipspharma.com

» **Merck Research Boston**
http://www.merck.com

» **Roche Palo Alto**
http://www.roche.com

» **Wyeth Research**
http://www.wyeth.com



CAREER OPPORTUNITIES

As a global leader in pharmaceutical drug discovery, sanofi-aventis' goal is to treat people with the very best therapeutic agents - *because health matters.* Our strong development pipeline is based upon scientific and medical innovation. We have a major commitment to CNS, our largest therapeutic department.

The **CNS** division is **expanding** its **psychiatric** and **neurological** disease areas. We currently have multiple career opportunities for innovative scientists at the **BS, MS and PhD** levels at our **Bridgewater, NJ location.**

Psychopharmacology: Focus on Schizophrenia

Job Code: SMA10442

Development and implementation of rodent and primate models of working memory and attention

Neurological Diseases: Focus on Multiple Sclerosis

Job Code: SMA10659

Development and implementation of in vivo and in vitro models of inflammation and repair

Molecular and Functional Neuropharmacology: Focus on Lead Identification and Optimization

Job Code: SMA10651

Development and implementation of biochemical and molecular assays for *in vitro* and *in vivo* pharmacology, in vivo proof of mechanism, and acute pharmacodynamics

At the heart of all that matters are people. Connected in purpose by career, life, and health. We at Sanofi-Synthelabo and Aventis Pharmaceuticals, members of the sanofi-aventis Group, are working together to focus on what is essential to us all–health.

Driven by a pioneering spirit, a strong set of core values and a mosaic of talent worldwide, we strive for success–in health. In doing so, we strengthen careers and enrich lives.

Discover your future with sanofi-aventis. Apply online today.

www.careers.sanofi-aventis.us

By embracing diversity of thought and culture, sanofi-aventis fosters positive, innovative thinking that will benefit people worldwide.

sanofi aventis

Because health matters

OPEN TO

DISCOVER

Within each discovery is a new future. That future begins with you.

At the Novartis Institutes, our Discovery Technology platforms meet the highest standards: cutting-edge technology and outstanding talent. Would you like to join our team of world-class scientists to deliver the next generation of lifesaving medicines?

We now have opportunities in biology, chemistry, discovery technologies, and pharmacology in the following disease areas: cardiovascular, diabetes, oncology, and infectious diseases. Our openings include positions in the following areas:

Assay Development	Enzymology	Lab Automation	Protein Purification
Biochemistry	Epigenetics	Mass Spectrometry	Proteomics
Bioinformatics	High-Throughput Screening	Molecular Biology	Robotics
Cell Signaling	Hit Discovery Management	Myogenesis	Scientific Computing and Statistics
Cell-Based Assay Development	Imaging	Neuroscience	Stem Cell Biology
Computational Chemistry	In Vivo Pharmacology	Oncology Pharmacology	Synthetic Organic Chemistry
Crystallography	Informatics Customer Support	Protein Expression	

To view descriptions of all open positions and to apply, visit www.nibr.novartis.com and follow the links to Careers and Job Opportunities.

Novartis is committed to embracing and leveraging diverse backgrounds, cultures, and talents to achieve competitive advantage.
Novartis is an equal opportunity employer. M/F/D/V

NOVARTIS
NOVARTIS INSTITUTES
FOR BIOMEDICAL RESEARCH

The New Pathway to Drug Discovery

www.nibr.novartis.com

Careers in Drug Discovery
Handling the Handoff

Scientists recruited straight out of academics to drug discovery and development teams can find the new approach confusing at best and daunting at worst. "College and university training is not adequately preparing students for what's going on in biopharmaceutical companies," says Bruno Battistini, vice president and chief scientific officer at IPS Pharma. "Even some Bachelor's degree courses in pharmacology are not aimed at critical aspects of drug development such as safety, pharmacotoxicology, pharmacokinetics, and pharmacodynamics. We need such expertise more than ever, and it gets even more difficult if you go into regulatory affairs and clinical development."


BRUNO BATTISTINI

Rarer Than Hens' Teeth

For young scientists, the good news is that life scientists who can demonstrate that they understand the various aspects of moving drugs along the pipeline and the transitioning between individual steps can write their own tickets. "People in these areas have great value," Battistini continues, "and they deserve to be treated with respect so that we can earn their trust." Donna Johnstone, director of discovery medicine for oncology and infection at AstraZeneca R&D, agrees. "Good recruits," she says, "are rarer than hens' teeth."

Any scientist who wants to work on drug discovery and development must demonstrate the ability to work effectively in teams. "Collaboration is essential at all stages of drug development," says Katherine Turner, vice president of validation biology at Biogen Idec, "but particularly at the research to preclinical development step where the focus moves from characterizing the efficacy of a candidate molecule to confirming its safety and completing the pharmacological characterization to get the candidate molecule ready for human clinical trials."

The emphasis on cooperation has a strong commercial basis. "It's absolutely critical to have very close collaboration so that you don't run the risk of discovery that has no value for the medical or commercial organizations," explains Jim Summers, divisional vice president for advanced technology, in Abbott's Global Pharmaceutical Research and Development division. Matthew Bell, director of discovery research strategy at Wyeth Research, extends that thought. "Right from the earliest stages of discovery and identification of a new gene or target," he says, "all the experiments are really focused on getting the compound or antibody to the clinic and asking all the questions you need to ask about it. So you have to talk to your development colleagues and your metabolism colleagues. You want to weed out those molecules that won't be successful and to maximize the value of the potential winners."


MATTHEW BELL

Johnstone points out another reason for ensuring smooth transitions. "The biggest problem in developing drugs is the time lost in the transition between discovery and development," she says. "This needs to be eliminated."


DONNA JOHNSTONE

Translational Medicine

The need for fast, efficient handoffs from discovery to development has stimulated new approaches and new terminology. Johnstone calls the diminishing barrier between discovery and development the "gray zone." Turner refers to it as "R2D." And industry insiders increasingly have coined a general phrase for the entirety of efficient transition. "We're working more on 'translational medicine' – translating discoveries from animals into humans," Bell explains.

Stein extends that definition. "Building a strong bridge between research and development and establishing traffic in both directions is the essence of translational medicine," he explains. "It allows us to be smarter in development based on what we learn in the laboratory and more innovative in the laboratory based on what we learn in the course of developing our medicines. I believe that continuing to optimize the quality of translational medicine is mission critical to translating the revolution in molecular understanding of disease that has unfolded over the last 50 years into great new medicine and better health for all people."

For many pharmas and biopharmas, translational medicine implies not only knocking down the walls between discovery and development but also creating discovery teams that morph into development groups, clinical trial organizations, and manufacturing divisions as promising molecules pass along the pipeline. "A link is made as quickly as possible between the candidate molecule and its role in human disease, often involving a close collaboration between the clinical groups and research," Turner notes. "As the project moves down the value chain, representatives from other downstream development functions join, including clinical, manufacturing, regulatory, and toxicology."

Those representatives possess skills different from those directly relevant to basic research. "You require a combination of people including members from clinical pharmacology, safety toxicity, process research, and regulatory affairs," says Merck's van der Ploeg. "And you want to bring in project management."

Equally important, members of modern discovery teams continue to keep in touch with their molecules as they move along the pipeline. Why? "They will work on the second and third generations of the same compounds, for intellectual property protection and for creating backup compounds," Battistini explains. "They also deal with the issue of reformulation of molecules for different routes of administration."

From Discovery to Development

Not surprisingly, different companies handle the transition from discovery to development in different ways. What they all have in common is the emphasis on continuity as promising molecules move along the pipeline.

As a young biopharma company, IPS Pharma had to set up operating procedures without tearing down previous approaches. "We have an integrated cascade that goes from discovery to clinical," Battistini says. "We have discovery, preclinical R&D, and clinical trials groups. We want these people to talk to each other. Once we have a novel technical platform, we move rapidly ahead with the

CONTINUED »

Will an understanding of inflammation and Lp-PLA$_2$ help Lynne prepare for a future free from atherosclerosis?

Multi-disciplinary Scientific Opportunities in Drug Discovery

Despite leading a healthy lifestyle and getting a clean bill of health from the family doctor, Lynne's future is still uncertain. Strokes and heart attacks can strike seemingly without warning or reason. Recent studies have identified that inflammatory processes may promote atherosclerosis – putting seemingly otherwise healthy people like Lynne at risk from cardiovascular events.

So could inflammation also hold the key to predicting the future? That question led our researchers to Lp-PLA2 (lipoprotein-associated phospholipase A2), usually bound to LDL, found in plaque and secreted by macrophages. Our researchers hypothesized that the enzymatic action of Lp-PLA2 yielded substances that caused inflammation, so-called inflammatory mediators. But first the linear sequence of the amino acids composing the structure of Lp-PLA2 had to be determined – potentially an arduous and daunting process.

Fortunately, our breakthrough work in genomics allowed us to accelerate this process by deducing the entire gene sequence based only on a mere fragment of 70 amino acids. Taking weeks instead of years, this means we are now much closer to developing drug therapies to combat the leading cause of death in industrialized societies. As a result, patients can look forward to a longer, healthier life, without having to worry about what the future might hold.

Across all therapeutic areas, our dedicated R&D scientists are making astonishing discoveries every day and paving the way for the development of life-saving therapies. But in order to achieve our full potential, we need people like you. In particular, we're looking for **Chemists, Cellular and Molecular Biologists, Pharmacologists, Immunologists and Biochemists** with the vision, ambition and dedication to further our understanding of diseases, evaluate potential therapeutics, and ultimately make these goals a reality.

To find out more about our research approaches to atherosclerosis and other disease areas, please visit our website at **http://science.gsk.com/careers/lynne.htm.**

GSK is proud to promote an open culture, encouraging people to be themselves and giving their ideas a chance to flourish. Developing talent through equality of opportunity, M/F/D/V

Together we can make life better.

gsk GlaxoSmithKline

Careers in Drug Discovery

Handling the Handoff


ROBERT STEIN

discovery, medicinal chemistry, and pharmacology teams. For a new platform, everything we've learned in development aimed at previous targets will apply to the second round, such as formulations. In addition, directors of the groups talk at weekly meetings, which helps to react to unforeseen events. We use the 'small is beautiful' idea to confront the 'bigger is better' of the past decade."

Merck undertook a slightly different startup last August when it opened its Research Laboratories in Boston "to give us the opportunity to work effectively with local biotechnology and academic institutions," van der Ploeg says. "We have all the disciplines to reach the point at which we have molecules ready for toxicology, after which they go into development. A core group brought in programs from other Merck research labs and we have started our programs in oncology. Later, we'll start an effort in neuroscience and Alzheimer's disease."

Biogen Idec relies on senior level employees to oversee its transitions. "We've created a new transition management team comprised of senior members of research and preclinical development to help project teams navigate through the R2D transition step," Turner says. "This management team meets regularly with late stage research project teams to help identify bottlenecks and provide assistance to the team with resources and advice to facilitate the project's staying on schedule. The transition management team forms approximately one year before the project is ready to move into development. By this stage the lead molecule has already been identified and characterized and the project team is in the final stages of preparing the molecule for entering preclinical development." This approach has a particular advantage, Turner adds, of facilitating early identification of molecules unlikely to make it to market.

Shepherding Molecules

Roche uses its research and development committee, a global group that guides development of its early pipeline, to accept promising compounds into early development. "Once a compound is accepted, it is shepherded by a multidisciplinary team with representation from both the discovery organization and development," Stein explains.

Scientific teams undergo their own transitions as molecules move from discovery to development. From the individual molecule's point of view, the scientists who handle it gradually change identities as it moves along the pipeline. "At Abbott, we try to start as early as we possibly can in the discovery process by adding development scientists into the process," Summers says. "We begin with a team that we've traditionally called discovery that morphs into what is essentially a development team." To enable that, the company complements the discovery team with new members who have expertise in such areas as biomarker discovery, toxicology, formulation, and commercial issues.

AstraZeneca takes a slightly different approach. "Imagine a relay race in which the second sprinter starts running while the first is coming round the corner with the baton," Johnstone says. "We have two teams and we have a handover, but it starts with a significant overlap. At the early stages, the discovery teams haven't got the luxury of understanding how to schedule doses, knowing what additional biology is required, and understanding patient identification. Those questions are picked up by our translational science group, which answers the key questions that the discovery scientists haven't been able to solve."

Wyeth has a similar setup. "We don't suddenly change from discovery people to development people; we gradually introduce our development colleagues," Bell says. "We have a dedicated team that's in between discovery and development. It consists in part of clinicians and in part of basic researchers. It focuses on biomarkers and other aspects of whether or not a drug will make the transition into humans." Like other pharmas, Wyeth does not regard a molecule's move into development as the end of the discovery story. "Discovery scientists stay with the project for support," Bell continues. "And senior staff stay with the project right up to and after it reaches the market."


JIM SUMMERS

Jacks of All Trades

What type of scientist do biopharmas seek to staff their discovery, development, and transition teams? "We need jacks of all trades," Johnstone asserts. "They need a good working knowledge of science and the technology platforms that can be used, from pharmacology, molecular biology, genomics, metabonomics, and pharmacokinetic analysis. It's important to have a broad range of experience." Summers echoes that point. "The old rules still apply," he says. "You need people who are very strong in their own primary disciplines but have the ability and desire to work in new areas and understand new areas. The vast majority of skill types that go into drug discovery are the same as we have had all along. What has changed is that the teams they are working on are much more multidisciplinary, and discovery scientists are staying with their molecules much longer."

Roche's Stein outlines the four types of expertise that pharmas and biopharmas expect teams that work on early development to possess: basic scientific understanding; pharmaceutical science; medical expertise related to specific diseases; and pharmaceutical development expertise. "Each of those," he continues, "is now a subject with its own expert practitioners."

Roche recruits mainly individuals with M.D.-Ph.D., M.D., or Ph.D. degrees, with or without previous experience in the pharmaceutical world – although Stein notes that "the best place to learn drug discovery and development is in the pharmaceutical industry." He adds that "we are also interested in qualified individuals with Master's or Bachelor's degrees."

AstraZeneca similarly prefers Ph.D.s and M.D.s, while being willing to hire individuals with alternative degrees who have good knowledge of research for its transition teams. "We're looking for generalists more than specialists – ones who can link lab work with CONTINUED »

scios

Focusing Science to ADVANCE MEDICINE



Scios Inc., headquartered in Fremont, California, a member of the Johnson & Johnson family of companies, is changing the way heart failure is treated. We are developing new and exciting therapeutics for cardiovascular disease, inflammatory disease and cancer. Our winning combination of integrated research, clinical experience, and passionate commitment to patients with unmet medical needs is making a difference in the management of disease. Innovation, collaboration and education are the cornerstones of Scios' culture.

We provide mentoring and career development opportunities throughout our organization. Our ability to grow as a company depends on finding and nurturing individuals with entrepreneurial spirit and passion to change the way patients are treated.

To support our continued growth, we seek candidates in the following areas:

CLINICAL RESEARCH

- Clinical Data Coder
- Clinical Research Associates
- Directors
- Lead - Clinical Data Mangement
- Manager, Supervisor - Clinical Supplies
- Managers
- Scientist

RESEARCH

- Associate Director, Bioanalytics
- Post Doc - Cell Biology
- Managers - Bioanalytics
- Medical Writers
- Research Associates, Formulations, HTS, Analytical Chemistry
- Scientists - Genomics, Informatics Toxicology, Analytical Chemistry, Pharmacokinetics

MEDICAL AFFAIRS

- Associate Director
- Directors
- Managers
- Medical Education
- Medical Writer
- Manager, Medical Education
- Senior Biostatistician, Lead, Associate Director - Biostatistics

REGULATORY AFFAIRS

- Associates
- Directors
- Managers

QUALITY CONTROL/ QUALITY ASSURANCE

- Analysts
- Specialist

For more information about us, to view a complete job description, and to apply online, please visit us at:

www.sciosinc.com

Equal Opportunity Employer

Johnson & Johnson

Careers in Drug Discovery

Handling the Handoff

applications in the clinic," Johnstone says. "We have recruited a number of people from the outside. They are generally cellular-based, but some have in vivo experience. I have had some chemists come to see me; I seriously consider them if they have some biological background. You have to bring in sufficient breadth of experience at least to dialogue with experts; you have to have some degree of 'street cred.'"


KATHERINE TURNER

A Catholic View

Abbott takes a catholic view of recruiting for its discovery and development teams. "We're recruiting all kinds of positions, including cell biology, molecular biology, and pharmacology, at all levels of training," Summers says. "We're always looking for very strong talent."

IPS Pharma also seeks to hire scientists at different levels in different fields. "In the discovery division we need people at technical levels, such as technicians to run in vitro assays, for example, and animal lab technicians," Battistini says. In many cases, scientists can make up for the lack of higher degree qualifications with working experience. "Sometimes a person with a Bachelor's degree in science with 10 or 20 years experience at the age of 40 has more knowledge than one with a Ph.D. and no experience in a pharmaceutical lab," he continues. "We try to organize a pyramid that will permit the younger scientists to benefit from the experience of the older ones. We also have a commitment to recruit bright, motivated M.Sc. and Ph.D. students from various state universities for which our directors participate as adjunct professors."

Wyeth has its own wide ranging recruitment goal. "We have opportunities for all levels of scientists," Bell says. "It depends on personal aspirations and career goals. Certainly for discovery, the more advanced training they have, the better. Postdoctoral training gives you the scientific credibility that can get you to the top in the industry. But in any research project you need a mix of senior and junior scientists. We also have people who can get their Ph.D.s on the job."

In terms of training, the company expects proven scientific skills and business skills. "We look for biologists with scientific excellence in pharmacology, cell biology, and other fields," Bell says. "We also have a large core technology team focused on gene chip arrays and other platform toolkits for all diseases; we look for people who have those skills. We have medicinal chemists and an increasing number of biotechnologists who work on antibody and recombinant therapies. We're increasing our interest in informatics. For translational medicine we have M.D.s who work in discovery alongside the therapy areas. And business operations is becoming increasingly important. You have to find people who think in a business way. They have often started as scientists and have found their way into the strategy side of the business."

Essential Skills

A feel for business is one of the abilities that AstraZeneca seeks when it selects scientists for what Johnstone calls "the underappreciated skill" of project management. "Effective project managers need the skills to keep a piece of work on track for clinical trials," she says. "They need good


LEX VAN DER PLOEG

communication skills to explain what they are doing to people in other disciplines and to get the key aspects of science understood by listeners in ways that are relevant to them. They need to be able to interpret clinical data and to avoid being frustrated if a clinical trial is less than perfect; we've selected out people who are perfectionists. The 80/20 rule has to apply in this area of science."

Merck, which plans to increase the number of scientists in its Boston research center from about 130 at present to 170 by the end of this year and 400 at the end of 2007, takes its own pragmatic view. "We want people with outstanding scientific records of accomplishment and great intellect," van der Ploeg says. "I'm less concerned with specific skills; the intellect determines what you can do over the next 10 or 20 years. We look for people with an absolute interest in drug development and, most important, an interest in joining the team. We want scientists willing to share responsibility."

Flexibility, collegiality, and communication skill are critical for any scientist who wants to enter drug discovery and development. "During our interviews, we try to evaluate the whole person – not just their technical or scientific expertise and training," Biogen Idec's Turner says. "For most positions we are looking for people with specific skills and talents. But we try to get to know the person and estimate how good a fit he or she will be in the new position. It's really important that the newly hired person will be happy and productive in the new job, and often their nonscientific skills are a critical element of their success. The drug discovery business is very dynamic and always changing. The best candidates will have good written and verbal communication skills and be flexible and able to adapt to changing environments."

Effective Communication

Bell amplifies that comment. "The scientist who likes to be left alone is not practical for us," he says. "The effectiveness of a scientist in communicating his or her idea impacts his or her chances of getting funded. Communicating clearly what you are doing, particularly to people with different backgrounds, is very important. Our hires have to have a strong scientific background. They must also have the ability to work in teams and cross-functional environments." Abbott's Summers puts the point more pithily. "To be successful in drug discovery, you need the best scientists; but you also need them to act as a team," he says. "They have to have the ability to work collegially and cooperatively, and to communicate well."

And what is the reward? Johnstone summarizes the virtues of the present-day method of handing off between discovery and development. "It really is a partnership between preclinical and clinical science," she says. "The joy in translational science is the tremendous synergism when it works well."

A former science editor of Newsweek, *Peter Gwynne (pgwynne767@aol.com) covers science and technology from his base on Cape Cod, Massachusetts, U.S.A.*

Others
call it research.

We call it changing the medical textbooks.

For over 29 years, Genentech has been at the forefront of the biotechnology industry, using human genetic information to discover, develop, commercialize and manufacture biotherapeutics that address significant unmet medical needs. A considerable number of the currently approved biotechnology products originate from or are based on our science. Genentech manufactures and markets multiple products in the United States, providing innovative treatments for cancer, heart disease, respiratory disease, growth disorders and other serious illnesses.

Genentech's research organization features world-renowned scientists who are some of the most prolific in their fields and in the industry. Genentech researchers have consistently published at a rate of 150+ papers per year and have secured more than 5,000 patents worldwide (with 5,300 more pending). Genentech's research organization combines the best of the academic and corporate worlds, allowing researchers not only to pursue important scientific questions, but also to watch an idea move from the laboratory into development and out into the clinic.

We are seeking:

Postdoctoral Fellows • Research Associates • Scientists

In the areas of:

- Angiogenesis
- Antibody Engineering
- Assay Technology
- Bioinformatics
- Biomedical Imaging
- Cancer Genomics
- Cell Biology
- Immunology
- Medicinal Chemistry
- Molecular Biology
- Molecular Diagnostics
- Molecular Oncology
- Pathology
- Protein Chemistry
- Protein Engineering
- Translational Oncology
- Tumor Biology

Meet with Genentech at the Science Career Fair August 10 at the Boston Convention Center, Boston, MA.

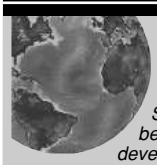
Genentech offers one of the most comprehensive benefits packages in the industry. Our culture emphasizes working hard, collaborating and sharing rewards. Our employees enjoy an environment that supports a work-life balance through benefits and programs that include sabbaticals, near-site childcare and concierge services. Join a company that attracts, retains and rewards the best and brightest employees. To view these and all our career opportunities, please visit **www.gene.com/careers.** Please use "Ad-Science" when a "source" is requested. Genentech is an equal opportunity employer.

Genentech

IN BUSINESS FOR LIFE

www.gene.com

EXTENDING & ENHANCING HUMAN LIFE.



Bristol-Myers Squibb is a world leader in oncology, infectious diseases, diabetes, immunology, neuroscience and cardiovascular research. To stay on top, we need to recruit new scientists who have the creativity to develop the medicines of tomorrow. For the next generation of leaders in pharmaceutical research, Bristol-Myers Squibb is an excellent place to work. You'll have the support of a culture that believes in making the unattainable a reality every day and the potential for career development and mobility across our entire drug development process.

Principal Scientist, Bioinformatics

An opportunity exists to join the Bioinformatics Group of the Bristol-Myers Squibb Pharmaceutical Research Institute as Principal Scientist. The successful candidate will join a diverse, scientifically and technically talented group of senior scientists whose mission is to advance targets and drug candidates in the R&D pipeline by providing an integrated view and interpretation of the wealth of modern genomic information.

Training and Experience

Experience Requirements: Ph.D. in the Natural Sciences or related disciplines is highly desirable. A minimum of 8 years' post-graduate experience, including 5 years working in life science environments, is necessary. Intimate knowledge of the pharmaceutical R&D process is an advantage. A high level of technical expertise in the following areas is desirable and in some cases essential: a high level of software development skill in a diverse and rapid-development environment, including high-level programming languages (currently Perl, Lisp, and Python), statistical languages, and relational databases; complete fluency in Linux environments (multiprocessor, cluster).

Tactical and Strategic Leadership

The successful candidate will join a diverse, scientifically and technically talented group of Senior Scientists whose mission is to advance targets and drug candidates in the R&D pipeline by providing an integrated view and interpretation of the wealth of modern genomic information. The candidate will set direction and vision for the BMS Bioinformatics platform and have the technical expertise to guide its implementation and evolution. Current areas of platform support include sequence analysis, genetics, transcript/protein, metabolite-based genomic profiling techniques, and high throughput cellular imaging methods. These genomic platforms are applied across the Institute in areas that encompass target identification and validation, drug selectivity and validation, predictive toxicology, drug safety, pre-clinical and clinical biomarker efforts.

Qualified applicants should email their resume to **recruit.pri@bms.com** with Job Code-Principal Scientist-Bioinformatics in the subject line.

Bristol-Myers Squibb is proud to be an equal opportunity employer, M/F/D/V.

Bristol-Myers Squibb Company
Pharmaceutical Research Institute

SK BIO-PHARMACEUTICALS

- **PHARMACOLOGY LEADER** CNS-related Diseases
- **SENIOR PHARMACOLOGIST** Parkinson's Disease
- **CLINICAL/MEDICAL DIRECTOR** Experience in CNS Preferred
- **REGULATORY AFFAIRS LEADER** Industry Regulatory Experience in Life Science Required

BS/MS/PhD candidates with pharmaceutical industry experience desired. Qualifying candidates should forward resume/CV with cover letter to:

Human Resources Department
SK Bio-Pharmaceuticals
140A New Dutch Lane
Fairfield, NJ 07004

Fax: (973) 227-4488
Email: cson@skbp.com
www.skbp.com

Anadys Pharmaceuticals, Inc. is a publicly traded biopharmaceutical company committed to advancing patient care by discovering, developing and commercializing novel small molecule medicines for the treatment of Hepatitis C virus, Hepatitis B virus and certain bacterial infections. On the heels of our deal with Novartis valued at up to $570 M, Anadys is poised to achieve its objective of building a leading franchise in anti-infectives.

At Anadys, the free exchange of ideas fosters a stimulating climate of teamwork, respect and creative agility. This strengthens our twin goals of both advancing patient care and your career. We achieve the first through scientific excellence; the second through ongoing training and promotion from within.

We are currently looking for the following team members:

Group Leader/Principal Scientist - Structural Chemistry
Group Leader/Principal Scientist - Medicinal Chemistry
Medicinal Chemists - all levels (Ph.D., M.S., B.S.)
Bioanalytical Scientists at all levels

Anadys is located in San Diego, a city whose active lifestyle will refresh your spirit for fun and inspire your creativity.

For full position descriptions or to apply, visit:
www.anadyspharma.com

ANADYS
PHARMACEUTICALS, INC.

SAN DIEGO'S BEST COMPANIES

VERTEX

Small Molecules. Huge Discoveries

Vertex Pharmaceuticals Incorporated is leading the way in small molecule drug discovery. By pioneering new, innovative, faster approaches to drug discovery with a focus on chemogenomics, our goal is to set the standard for pharmaceutical research and development in the 21st century. We currently have the following opportunities in our **Cambridge, MA** location.

RESEARCH SCIENTISTS

PROTEIN BIOCHEMISTRY - 4050-01B
IN VIVO PHARMACOLOGY - 4130-01B
INFECTIOUS DISEASES - 4110-01B
KNOWLEDGE MANAGEMENT/CIA - 4430-02B
BIOMARKER TECHNOLOGIES - 4200-01U

We provide a highly stimulating working environment coupled with a high level of professional and intellectual challenge. In addition to competitive salary and benefits, we also offer equity participation and participation in a stock purchase program. No agencies, please. Apply online at www.vrtx.com. EOE

VERTEX

We Enjoy a Big Lead in Small Molecules.

Roche

Pharmaceuticals

Lab Head in Metabolic Diseases

Who we are

Headquartered in Basel, Switzerland, Roche is one of the world's leading research-focused healthcare groups in the fields of pharmaceuticals and diagnostics. As a supplier of innovative products and services for the early detection, prevention, diagnosis and treatment of disease, the Group contributes on a broad range of fronts to improving people's health and quality of life. Roche is a world leader in diagnostics, the leading supplier of medicines for cancer and transplantation and a market leader in virology.

The position

You will be expected to lead a team engaged in discovery and validation of new targets and pathways for the treatment of Type 2 Diabetes/Metabolic Diseases. During the challenging process of developing new antidiabetic drugs, you will interact with a multidisciplinary team composed of excellent scientists with a proven track record in drug discovery and basic research.

Who you are

You should have an MD or a PhD or equivalent in biochemistry with the relevant training in Type 2 Diabetes/Metabolic Diseases; a strong background in drug discovery in these areas and at least five years' experience in pharmaceutical research. Good communication skills and the ability to work effectively both with team members and across disciplines to collectively drive projects forward are essential.

Who to contact

If your experience and interests match the above profile and you would like to apply for these challenging position, please forward your application, with full supporting documentation, to: F. Hoffmann-La Roche Ltd, Ralph Gysin, PSHB-4, building 52/202, P.O. Box, CH-4070 Basel, quoting reference: Gr9864. For further information, please contact Dr Elena Sebokova, email: elena.sebokova@roche.com

Positions @ NIH

THE NATIONAL INSTITUTES OF HEALTH

Interview/Hire the World's Best Scientists

Reach innovative young scientists at the forefront of their fields

Attend the Job Fair for NIH Postdoctoral, Research, and Clinical Fellows

Date: October 20, 2005
Time: 10:30 am - 3:00 pm
Place: Natcher Conference Center
National Institutes of Health
Bethesda, Maryland

Access 3800 doctoral scientists and clinicians in training at the NIH

Register by September 15th at: **www.training.nih.gov/jobfair**

Office of Intramural Training and Education

Postdoctoral, Research and Clinical Fellowships at the National Institutes of Health

www.training.nih.gov/pdopenings

www.training.nih.gov/clinopenings

Train at the bench, the bedside, or both

Office of Intramural Training and Education
Bethesda, Maryland 20892-0240
800.445.8283

NIDDK

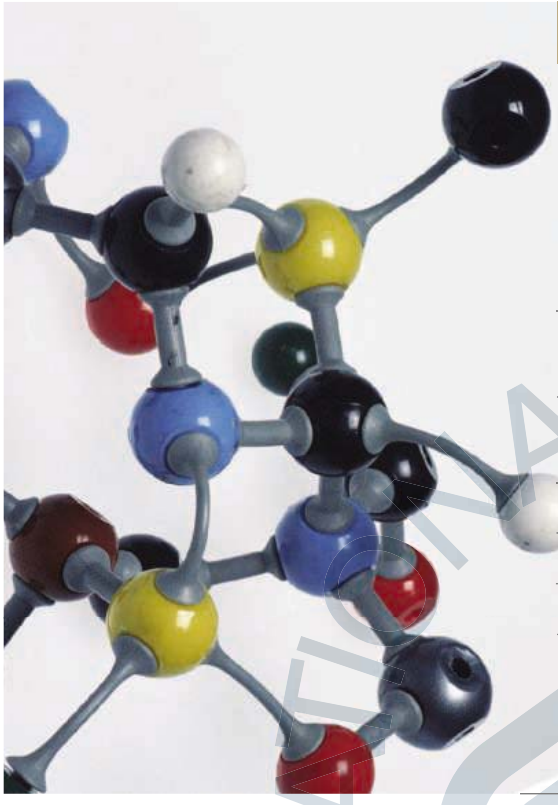
Molecular Genetics of Signal Transduction/Tumorigenesis

We have helped discover two genes causing hereditary endocrine cancers (MEN1 and HRPT2). Our efforts are directed at function of menin, the encoded protein from the MEN1 tumor suppressor gene. With protein-protein interaction methods, we have identified four menin partners. Our lab also collaborates extensively with other labs in the NIDDK, NCI and NHGRI to study the function of menin in Drosophila and in knockout mice. This interaction involves creativity and lab expertise with cutting-edge methods. **This position is available September 1.** Interested individuals not more than 5 years after the most recent doctoral degree and with excellent experience in a related field should send a CV plus contact data for three references to:

Stephen Marx MD
NIH
Bldg. 10 Rm. 9C-101
Bethesda, MD 20892

Fax 301-496-0200; (StephenM@INTRA.NIDDK.NIH.GOV). Salary is at the NIDDK scale and commensurate with research experience and accomplishments.

WWW.NIH.GOV



Postdoctoral Research Training at NIH

- Launch a career to improve human health
- Work in one of 1250 of the most innovative and well-equipped biomedical research laboratories in the world
- Explore new options in interdisciplinary and bench-to-bedside research
- Develop the professional skills essential for success
- Earn an excellent stipend and benefits
- Click on www.training.nih.gov

Office of Intramural Training and Education

National Eye Institute

Retinal Pigment Epithelium (RPE) Mediated Drug Delivery Across the Blood – Retina Barrier

A post-doc/scientist position is available in the Section on Epithelial and Retinal Physiology and Disease, Division of Intramural Research, NEI/NIH to investigate the membrane and intracellular mechanisms that mediate the transport of novel therapeutic molecules & drugs across the sclera, choroid and RPE. The RPE is a monolayer of epithelial cells in the back of the eye that separates the choriocapillaris and the neural retinal and is part of the blood-retinal barrier. It has multiple barrier and transport functions that serve to maintain the health and integrity of retina and choroidal blood supply (Maminishkis, et al., Investigative Ophthalmology & Visual Science, v43,pg.3555, 2002). The RPE is a site of deterioration in age-related macular degeneration and therefore an active area of research for potential treatment strategies against this disease. Applicants must have a Ph.D. or M.D., and a strong background in more than one of the following areas: membrane physiology, pharmacology, biophysics, bioengineering, or physical chemistry. Previous experience working on drug delivery and targeting, especially across the blood-brain barrier is desirable along with experience in histology, cell culture and molecular and cell biology. This position will allow access to a wide variety of scientific and career development opportunities available at NIH. The salary range begins at $42,600 and is commensurate with experience.

Please send CV, description of research interests, names and addresses of three references to:**Jeff Hammer, c/o Sheldon S. Miller, SERPD, DIR, NEI, NIH Building 10/Room 10B04, 10 Center Drive, Bethesda MD 20892-1857, Email: SERPD@NEI.NIH.GOV Fax: (301) 451-2040.**

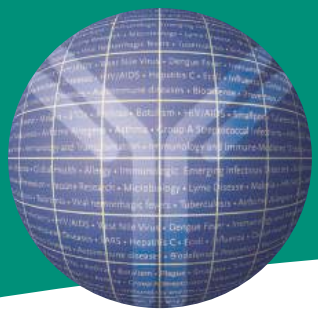
National Eye Institute

Regulation of Water Movement Across Epithelia

A postdoctoral/scientist position is available immediately in the Section on Epithelial and Retinal Physiology and Disease, Division of Intramural Research, National Eye Institute (NEI), National Institutes of Health (NIH), Department of Health and Human Services (DHHS) located in Bethesda, MD to study molecular, intracellular, and plasma membrane mechanisms that regulate cell volume, pH, Ca2+, K, Na, and water movement across epithelia. For in vitro experiments projects will involve the use of conventional and double-barreled microelectrodes, fluorescence imaging, and capacitance probe techniques for measuring fluid transport. In vivo experiments involving OCT, angiography, and ERGs will be used to test the efficacy of therapeutic interventions in animal models of disease. Applicants must have a Ph.D. or M.D., a strong background in physiology, cell biology, biophysics, or bioengineering and less than 5 years of postdoctoral experience. Experience in imaging, animal models, histology and cell culture is also desirable. This position will allow access to a wide variety of scientific and career development opportunities available at NIH. The salary range begins at $42,600 and is commensurate with experience.

Please send CV, description of research interests, names and addresses of 3 references to:

Jeffrey Hammer, c/o Dr. Sheldon S. Miller, SERPD, DIR, NEI, NIH, Building 10/Room 10B04, 10 Center Drive, Bethesda MD 20892-1857. Email: SERPD@NEI.NIH.GOV, Fax: (301) 451-2040



Health Research in a Changing World

Fighting Diseases and Improving Lives

DEPUTY DIRECTOR

Immediate Office of the Director
National Institute of Allergy & Infectious Diseases
National Institutes of Health
Department of Health & Human Services

The National Institute of Allergy & Infectious Diseases (NIAID) is seeking exceptional candidates for the position of Deputy Director, NIAID, to provide leadership with the Director for a national research program to understand, treat, and prevent infectious, immunologic, and allergic diseases throughout the world.

A $4.4 billion organization, the NIAID supports well over 100 major research programs and initiatives within three broad, distinct mission areas: Biodefense research ($1.7 billion/year), AIDS research ($1.4 billion/year) and the NIAID traditional research mission of immunologic and infectious diseases ($1.3 billion/year). NIAID is the lead Institute at NIH and for the U.S. Government for these three broad research missions and provides leadership and support to research activities in over 800 organizations principally in the United States, but also world-wide in more than 80 countries.

Within the last few years, the scope and complexity of the NIAID research operations have expanded dramatically to respond to emerging and immediate public health challenges and to meet the urgent mandates of the President and Congress. These include the need to develop an AIDS vaccine to combat the global AIDS pandemic; develop drugs, vaccines, and diagnostics to treat and prevent emerging infectious diseases such as Severe Acute Respiratory Syndrome (SARS) and the West Nile Virus; and develop medical countermeasures to combat bioterrorism attacks. In addition, scientific opportunities facilitated by technological advances and progress in the core NIAID scientific disciplines of microbiology and immunology, as well as progress in new areas of pathogen and human genomics, are enabling the development of new treatments, vaccines, and diagnostic tests that will improve the health of people in America and around the world.

The NIAID has clinics and laboratories located in Bethesda, Frederick, and Rockville, Maryland, as well as in Hamilton, Montana.

This position offers a unique and challenging opportunity for the right individual to assist the Director in providing strong and visionary leadership to an organization dedicated to uncovering new knowledge and technology. Specifically, the position will coordinate all activities related to the mission and function of the institute; execute all policies, and allocate all resources to carry out these policies. Also, the Deputy will represent the Director and serve as the Director in his absence.

QUALIFICATIONS:
Applicants must possess an M.D., Ph.D., or equivalent degree and senior-level research experience and knowledge of research programs in areas consistent with the mission of the NIAID. Preference will be given to those known and respected within their profession, both nationally and internationally, as distinguished individuals of outstanding scientific competence and those that possess a record as a senior scientific administrator/executive leader.

Administrative/executive skills will be assessed based on the following qualifications:

1. **Leading Change**—Please describe your leadership skills in developing and implementing an organizational vision, which integrates key national and program goals, priorities, values and other factors. Inherent to it is the ability to balance change and continuity, to continually strive to improve customer service and program performance within the basic Government framework, to create a work environment that encourages creative thinking, and to maintain focus, intensity and persistence, even under adversity.

2. **Leading People**—Please describe your leadership skills in designing and implementing strategies, which maximize employee potential and foster high ethical standards in meeting the organization's vision, mission and goals.

3. **Results Driven**—Stresses accountability and continuous improvement. Please describe your ability to make timely and effective decisions and produce results through strategic planning and the implementation and evaluation of programs and policies. Describe your ability to develop long-term strategic plans and translate them into tactical plans and operational activities.

4. **Business Acumen**—Please describe your skills and abilities in acquiring and administering human, financial, material, and information resources in a manner which instills public trust and accomplishes the organization's mission, and to use new technology to enhance decision making.

5. **Building Coalitions/Communication**—Please describe your experience in explaining, advocating, and expressing facts and ideas in a convincing manner, and negotiating with individuals and groups internally and externally. It also involves the ability to develop an expansive professional network with other organizations, and to identify the internal and external politics that impact the work of the organization.

APPLICATION PROCESS: Please submit a curriculum vitae, bibliography and narrative addressing the five administrative/executive qualifications above. Please limit responses to one page per "qualification". The narrative should demonstrate the necessary level of management skills, characteristics, qualities, specialized knowledge, and technical competence that would indicate successful performance in the position. Examples should be clear and concise and emphasize your level of responsibilities, scope and complexity of the programs managed, program accomplishments with results of your actions, policy initiatives, and level of contacts.

Applications to: Ms. Lisa Poindexter-Steed, Office of Administrative Management & Operations, NIAID, Building 31, Room 7A18; 31 Center Drive, MSC 2520, Bethesda, Maryland, 20892-2520 and reference announcement number **DD-05-01**. The application review process will begin September 9, 2005. Applicants must be a U.S. citizen. Salary is commensurate with experience and a full package of benefits is available including retirement, health and life insurance, long term care insurance, leave and savings plan (401K equivalent). Direct inquiries to : Ms. Poindexter-Steed via email: lsteed@niaid.nih.gov or at 301-594-3964. Information regarding the Institute is available on our website at www.niaid.nih.gov . All information provided by applicants will remain confidential and will only be reviewed by authorized officials of the NIAID.

DIRECTOR
GEMINI OBSERVATORY

The Association of Universities for Research in Astronomy (AURA) seeks a new Director for the Gemini Observatory, with the aim of filling this position in early 2006. The Gemini Observatory is an international partnership to operate twin 8.1-meter telescopes, one on Mauna Kea in Hawaii, and the other on Cerro Pachón in Chile. The partners include the United States, United Kingdom, Canada, Chile, Argentina, Australia, and Brazil. AURA manages the Gemini Observatory under the auspices of the International Gemini Board and the U.S. National Science Foundation (NSF) as its executive agency. Gemini currently has a staff of approximately 160, including scientists, engineers, technicians and administrative personnel.

The Gemini Director is responsible to the Gemini Board, through the Executive Agency and AURA, for the overall operation of the Observatory. The Director interacts directly with representatives of the Executive Agency and the partner countries. The Director leads a scientific, technical, and administrative staff to carry out the mission of the Observatory and to conduct Gemini-related research.

Candidates for Director should have demonstrated strong scientific leadership and have an established record of research achievement, preferably in astronomy or a closely related field. Candidates should also have demonstrated talent for administration and management, combined with skill in institutional and international relations.

Applications will be accepted until the position is filled. All applications received by **September 30, 2005** will be given full consideration. Applications must include: a curriculum vitae, information on relevant experience and accomplishments, the candidate's vision for the evolution of the Gemini Observatory, and the names of three professional references. Please send applications to:

Gemini Director Search Committee
c/o AURA
Suite 350
1200 New York Avenue NW
Washington, DC 20005

The Search Committee will hold all applications in confidence. Questions related to this search should be directed to **Dr. Robert McLaren**, Chair of the Gemini Director Search Committee at **mclaren@ifa.hawaii.edu**. Information and updates regarding this search are available on **www.aura-astronomy.org**.

Women and minorities are encouraged to apply. AURA is an EOE/AA/F/D/V Employer.

Scholars in Oncologic Molecular Imaging

Postdoctoral Training in Molecular Imaging of Cancer University of California Los Angeles, USA

The UCLA Scholars in Oncologic Molecular Imaging (SOMI) Program will graduate 'leaders of tomorrow' in the molecular imaging field. This rapidly growing area combines the disciplines of cell/molecular biology, chemistry, biomedical physics, biomathematics, pharmacology, imaging sciences, and clinical medicine to advance cancer research, diagnosis and management. SOMI fellows will conduct innovative research in cancer imaging under the supervision of two faculty mentors from complementary fields, in a comprehensive, integrated, three-year program. Support is available for salaries (which can be supplemented by mentors) as well as supplies and travel.

- **Applicants must be US citizens or permanent residents.**
- Further information about the UCLA SOMI program, application forms, and deadlines can be found at: *www.crump.ucla.edu/public/somi*
- Inquiries to SOMI Application Coordinator by telephone: 310-825-4903 or email: *ecorrin@mednet.ucla.edu*

WEILL CORNELL MEDICAL COLLEGE IN QATAR

FACULTY POSITIONS

In a pioneering international initiative, Weill Medical College of Cornell University established the Weill Cornell Medical College in Qatar (WCMC-Q) through a unique partnership with the Qatar Foundation for Education, Science and Community Development. Located in Doha, Qatar, and in its fourth year of operation, Weill Medical College of Cornell University seeks candidates for faculty positions to teach in Doha in:

• Cell Biology • Cell Physiology • Genetics • Molecular Biology • Molecular Pharmacology • Pharmacology • Physiology

Following a two-year Pre-medical Program, the inaugural class has now completed the first year of the traditional four-year education program leading to the Cornell University M.D. degree, which they will receive in May 2008. The medical program at WCMC-Q replicates the admission standards and the innovative problem-based curriculum, which includes, among other things, integrated, multidisciplinary basic science courses that are the hallmark of the Weill Medical College of Cornell University.

Faculty, based in Doha, will be expected to teach their specialty and to contribute to the academic life of the Medical College. This unique program provides the successful applicant with the opportunity to leave his/her mark on a pioneering venture. A state of the art research program, to be housed in WCMC-Q and focused on genetics with an emphasis on diabetes, obesity, hypertension and metabolic bone disease will be initiated within the next year. Teaching and research facilities are situated within a brand new building designed to Cornell specifications and located in Education City in Doha amongst other American universities.

All faculty members at WCMC-Q are appointed by the academic departments at Weill Medical College of Cornell University.

Further details regarding the WCMC-Q program and facilities can be accessed at: www.qatar-med.cornell.edu.

Candidates should have a M.D., Ph.D. or M.D./Ph.D. or equivalent terminal degree. Salary is commensurate with training and experience and is accompanied by an attractive foreign-service benefits package. Applicants should submit a letter of interest outlining their teaching and research experience and curriculum vitae to:

facultyrecruit@qatar-med.cornell.edu

***Please quote *Faculty Search #05-016-sci* on all correspondence**

Weill Medical College of Cornell University is an equal opportunity, affirmative action educator and employer.

The screening of applications will begin immediately and continue until suitable candidates are identified.

Tenure Track Position in Microbiology

Indiana University School of Medicine - Northwest is seeking applicants for a tenure-track position as Assistant/Associate Professor. The successful candidate will participate in a problem-based curriculum, research and service missions at an innovative medical school housed in new facilities (**http://shaw.medlib.iupui.edu/nwcme/nwcme.html**). Strong commitment to teaching medical Microbiology is required. The candidate will be expected to develop and maintain an innovative, externally funded research program. Preference will be given to candidates with ability to attract extramural funding and whose research expertise complements ongoing programs. A competitive start-up package is offered. Applicants must have a Ph.D. and/or M.D. or equivalent degree.

Send C.V., description of research, statement of teaching philosophy and names and contact information of three references to:

W. Marshall Anderson, Ph.D.
Search Committee Chair
IU School of Medicine NW
3400 Broadway
Gary, IN 46408

E-mail: **wanders@iun.edu**

EO/AA Employer, M/F/D.

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
National Institutes of Health

POSTDOCTORAL FELLOWSHIP (Ph.D./M.D.) OPPORTUNITIES

The National Cancer Institute offers numerous postdoctoral fellowship opportunities in a large variety of science disciplines (chemistry, biochemistry, bioinformatics, biology, biostatistics, cancer biology, cell biology, epidemiology, genetics, HIV research, immunology, microbiology, molecular biology, nuclear radiochemistry, nutrition, optical probe chemistry, pathology, pharmacology, virology, etc.).

Fellowship opportunities can be viewed on our training and employment Web site "StarCatcher" **http://generalemployment.nci.nih.gov**. We recommend that you post your resume in either job category "Postdoctoral Fellowship (U.S. citizens and premanent residents)" or "Postdoctoral Fellowship (foreign visiting fellows)" for viewing by our principal investigators. Then use the links to our research divisions to apply for current positions and communicate directly with the principal investigators. The **Center for Cancer Research** (NCI's largest clinical and basic science research division) lists multiple fellowship opportunities on their link and provides the opportunity to search their index of branches/labs/programs to find areas of research of particular interest to you. The **Division of Cancer Epidemiology and Genetics** provides an online application for fellowships in molecular, nutrition, radiation and genetic epidemiology. The **Division of Cancer Prevention** offers an online application process for fellowship opportunities in cancer prevention.

NCI facilities located in Bethesda, Rockville, Gaithersburg and Frederick Maryland, present a professional environment and possess the best-funded and equipped laboratories in the United States. As the largest institute within the National Institutes of Health, NCI provides postdoctoral fellows the opportunity to interact with scientists from a wide range of life/medical sciences, and to attend lectures given by international renowned scientists. Stipend range $40,900 to $66,100 commensurate with experience. Standard self and family health insurance is provided and high-option coverage is available.

Open to graduating doctorate degree (Ph.D. and/or M.D.) students and current postdoctoral fellows with less than 5 years postdoctoral experience. U.S. citizenship, permanent residency (green card), or current authorization (F-1 or J-1 visa) for training in the United States is required.

Apply online at **http://generalemployment.nci.nih.gov**

DHHS, NIH and NCI are EQUAL OPPORTUNITY EMPLOYERS

U.S. Environmental Protection Agency
Office of Research and Development
National Center for Computational Toxicology

VACANCIES IN BIOINFORMATICS AND HIGH THROUGHPUT SCREENING

EPA is seeking qualified individuals to fill three vacancies in the newly formed National Center for Computational Toxicology (NCCT). The NCCT is housed within the Office of Research and Development and is located in Research Triangle Park, NC. More information on EPA's computational toxicology program is available at: **http://www.epa.gov/comptox/** The NCCT provides scientific leadership, understanding and tools related to the application of mathematical and computer models to technologies derived from computational chemistry, molecular biology and systems biology in order to improve the Agency's data reporting requirements, priority setting approaches to understanding chemical toxicity, and risk assessment approaches. The major challenge of the research program is to improve the predictive capabilities of the methods, models and measurements that constitute the input materials to the computational models.

Major duties for these three positions include:

- **Lead Bioinformaticist (Research Biologist GS-14). RTP-DE-2005-0143**; Serves as senior bioinformatics advisor to the NCCT Director; conducts independent research and develops new statistical and bioinformatics methods to support the use of genomic information in risk assessment; provides lead technical oversight of STAR Center for Environmental Bioinformatics; and conducts data analysis and develops computer programs for data mining.
- **Bioinformaticist (Research Biologist GS-12/13). RTP-DE-2005-0144**; Conducts independent research and develops new statistical and bioinformatics methods to support the use of genomic information in risk assessment; conducts data analysis and develops computer programs for data mining; and provides training for EPA researchers in the use of bioinformatics tools.
- **Lead High-Throughput Screening (Research Biologist/Toxicologist GS-14). RTP-DE-2005-0145**; Provides leadership and guidance in the broad areas of prioritization, categorization and first level screening of chemicals for testing and develops a broad ranging research program in this area; serves as lead spokesperson in ORD in dealing with high throughput screening assays and other tools of modern molecular biology and chemistry, including genomic, proteomic and metabonomic technologies applied to chemical prioritization and categorization issues; and develops and provides oversight of extramural contracts.

These are full-time, permanent positions. U.S. citizenship is required and applicants must meet U.S. Office of Personnel Management qualification requirements including specific educational course work. The selected applicant will be eligible for full benefits including relocation expenses, health and life insurance, retirement, recruitment/signing bonus, and vacation and sick leave.

How to Apply: The official announcement and instructions on how to apply will be available at: **www.epa.gov/ezhire** under the announcement numbers referenced above. Select "Apply for Jobs." If you are registered in the Ezhire @EPA system, access the vacancy announcement through "Registered Users," otherwise, select "New Users" and complete the registration process. The vacancy announcement is located in the section "Any Qualified Applicant." **These vacancies will be open July 25, 2005 and close August 19, 2005**. All application materials must be submitted online by the closing date.

For additional information, please contact: **Ms. Barbara Howard** at **(800) 433-9633** or via email at **howard.barbara@epa.gov**.

The U.S. EPA is an Equal Opportunity Employer.

The Children's Hospital at SCOTT & WHITE

College of Medicine
The Texas A&M University System Health Science Center

Endowed Chair
Pediatric Cardiovascular Research
Scott & White Health System
Texas A&M University System Health Science Center
College of Medicine

The **Children's Hospital at Scott & White** and **The Texas A&M University System Health Science Center College of Medicine** are seeking a nationally recognized research scientist as the first holder of the Josephine Ballard Endowed Chair in pediatric cardiovascular research. Applicants should be accomplished investigators (Ph.D., M.D. or M.D./Ph.D.) at the associate or professor level with current federal grants and a proven track record in cardiovascular basic, clinical, and/or translational research. The successful candidate will join an expanding faculty within a large academic healthcare system. The chair holder will play a critical role in directing and expanding research activities in pediatric cardiovascular disease, in close collaboration with investigators in the Cardiovascular Research Institute and other local, national and international experts in cell biology, genomics and proteomics.

The Children's Hospital at Scott & White serves a large clinical base throughout Central Texas. There are outstanding clinical practice and laboratory facilities on campus that perform state of the art molecular and cellular biology techniques, flow cytometry, proteomics and genomics as well as biostatistical support services. Animal laboratory facilities include areas to perform medical and surgical procedures. Laboratory space and an appropriate start-up package for the chair holder will be provided. The Scott & White Healthcare system is one of the largest multi-specialty integrated delivery systems in the nation. Scott & White is the primary clinical and hospital teaching campus for the College of Medicine. Academic appointments at the associate and professor level through the College of Medicine are commensurate with qualifications and experience.

Interested candidates should send a copy of their curriculum vitae, letter addressing their qualifications and a list of 3 individuals who can provide references to: **Don P. Wilson, M.D., Chair, Search Committee for Josephine Ballard Centennial Chair in Pediatric Cardiovascular Research; Chairman, Department of Pediatrics, 2401 South 31st Street, Temple, Texas 76508, 254-724-4363, fax 254-724-1938, email: dwilson@swmail.sw.org**

Scott & White is an equal opportunity employer. For more information regarding Scott & White and The Texas A&M University System Health Science Center College of Medicine, please log onto: www.tamu.edu and www.sw.org.

PCOM

ASSISTANT PROFESSOR BIOCHEMISTRY/ MOLECULAR BIOLOGY

The Department of Biochemistry/ Molecular Biology at Philadelphia College of Osteopathic Medicine has a tenure-track **Assistant Professor** position available. The successful candidate for this position is expected to establish a strong research program, seek external funding, and contribute to the overall educational goals of the department, including teaching in medical and graduate programs. Research collaborations with faculty at PCOM and/or neighboring institutions are encouraged.

Requirements: Must have a doctoral degree in Biochemistry or a related field, with at least two years of postdoctoral experience and the potential to initiate and maintain a vigorous, independent research program. Must have good written and verbal communication skills; teaching experience in the field of biochemistry preferable.

Application should include a complete Curriculum Vitae, relevant reprints, a brief summary of proposed research plans, and letters of reference from three scientists familiar with their work. Send this information to:

PCOM
4190 City Avenue
Human Resources Department
Philadelphia, PA 19131
Fax: 215-871-6505
E-mail: hr@pcom.edu

EEO

www.pcom.edu

esf

Chair of Environmental and Forest Biology

College of Environmental Science and Forestry-SUNY, Syracuse, NY

The Faculty of Environmental and Forest Biology (EFB) seeks a distinguished researcher and educator in a discipline of environmental biology to be Chair and a tenured, calendar year Professor. EFB is the largest department in the College of Environmental Science and Forestry, a premier educational institution with emphasis on the science, design, engineering, policy and management of natural and built environments. A hallmark of EFB is its breadth, ranging from molecules to ecosystems, and extensive field programs that emphasize working in diverse regions and environments.

The Chair will lead and administer an internationally recognized program in teaching, research and public service. Excellent communication and interpersonal skills will aid in fostering collaboration within this academic setting. Undergraduate students obtain B.S. degrees in either the broad area of environmental biology or more specialized areas in aquatic and fisheries science, biotechnology, conservation biology, forest health, natural history and interpretation, and wildlife science. Graduate study areas (MPS, MS and PhD) include conservation biology, chemical ecology, ecology, entomology, environmental interpretation, environmental physiology, fish and wildlife biology and management, forest pathology and mycology, and plant science and biotechnology.

Further information on EFB and the position can be found at **www.esf.edu/efb** and **www.esf.edu/hr/search**, respectively. For nominations submit a letter with the nominee's name, title, business address, telephone number and email address. Application letters should include a description of academic qualifications, leadership qualities, administrative experience and educational philosophy. Applications will be accepted until the position is filled, but evaluation of applications will begin on Sept. 1, 2005. Send nominations and applications directly to: **Dr. Myron J. Mitchell, Chair of the Search Committee, 241 Illick Hall, SUNY-ESF, 1 Forestry Drive, Syracuse, NY 13210-2778; Tel. 315-470-6765, Fax 315-470-6996, Email:mitchell@syr.edu.**

SUNY-ESF is an Equal Opportunity/Affirmative Action employer.

COLUMBIA UNIVERSITY
Biomechanics

The Department of Biomedical Engineering in the Fu Foundation School of Engineering and Applied Science at Columbia University is seeking to fill one tenure-track faculty position at the Assistant or Associate Professor level but exceptionally qualified candidates will be considered for higher-level position. Applicants should have a doctoral degree in the area of biomedical engineering or a closely related discipline, and should be prepared to establish a vigorous and independent research program in any of the broadly defined areas of biomechanics: functional tissue engineering, biological systems modeling, molecular modeling, cellular or molecular biomechanics, biomechanics of growth and remodeling, biofluid mechanics, tissue mechanics, computer and robot-assisted surgery, and/or bioMEMS.

Applicants should send a complete curriculum vitae, three publication reprints, a statement of research interests, and names of four references to:

Professor X. Edward Guo
Chair of Biomechanics Search
Department of Biomedical Engineering
Columbia University
351 Engineering Terrace, Mail Code 8904
1210 Amsterdam Avenue
New York, NY 10027

Applications will be accepted until the position has been filled.

Columbia University is an affirmative action/equal opportunity employer. Women and minorities are encouraged to apply.

Department of Health and Human Services
National Institutes of Health (NIH)
National Heart, Lung, and Blood Institute (NHLBI)

The Division of Heart and Vascular Diseases (DHVD) seeks exceptional candidates for the position of Director, Heart Research Program, to provide leadership for a national research program in diseases of the heart. Acting with Congressional mandates, an annual budget exceeding $1 billion dollars, and a staff of 62, the Division oversees ambitious grant, contract, and career development programs targeting areas such as heart attack and heart failure, sudden cardiac death, high blood pressure, and stroke. Other areas of cutting-edge research include genomics and proteomics, nanotechnology and bioengineering, and cell-based therapeutics and gene therapy.

Within the Division, the Heart Research Program (HRP) supports basic, applied, and clinical research on diseases of the myocardium, from embryonic life to adulthood. Key areas include cardiac development and congenital heart disease, ischemic heart disease, heart arrhythmias and electrical abnormalities, heart failure, and cardiomyopathies. The Program Director provides strong and visionary scientific leadership and, along with the 15 HRP staff members, works with scientists all over the world in academia, industry, other government agencies, and non-government organizations, in order to diminish the toll of heat disease through a variety of activities:

- identify areas of scientific priority and need and develop new research efforts through grant and contract programs to address those needs
- oversee basic and clinical research and clinical trials funded by research grants and contracts
- translate new knowledge from bench to bedside, medical practice, and public health.

Applicants must possess an M.D. or Ph.D. or equivalent degree and senior-level research experience and knowledge of research programs in one or more scientific areas related to cardiovascular diseases. They should be known and respected within their profession, both nationally and internationally, as distinguished individuals with outstanding scientific, managerial, and communication skills.

Salary is commensurate with experience and a full package of Civil Service benefits is available, including retirement, health and life insurance, long term care insurance, paid vacation and sick leave, flexible spending accounts for health and dependent care, family-friendly leave programs, and savings plan (401K equivalent). (A complete description of benefits is located at **http://www.opm.gov/insure/health/new_employees.asp**). Additional Physician Benefits: supplemental physician incentive payments to Federal salary; part-time clinical assignments at NIH or Washington-Baltimore locations are possible; Extramural Loan Repayment Program for educational debt for qualified employees. Applicants must be US citizens. The position is located in Bethesda, Maryland. CV, bibliography, and two letters of recommendation must be received by **September 1, 2005**. Application package should be sent to:

Dr. Sonia Skarlatos, Deputy Director
Division of Heart and Vascular Diseases
National Heart, Lung, and Blood Division
6701 Rockledge Drive MSC 7940
Bethesda, MD 20892-7990

For further information, please contact Dr. Skarlatos by email: **Skalrats@nhlbi.nih.gov** or telephone 301-435-0477.

With nationwide responsibility for improving the health and well being of all Americans, the Department of Health and Human Services oversees the biomedical research programs of the NIH. The NIH encourages the application and nomination of qualified women, minorities, and individuals with disabilities.

DHHS and NIH are Equal Opportunity Employers

Leibniz-Institute for
Natural Product Research
and Infection Biology
-Hans-Knoell-Institute - Friedrich-Schiller-Universität Jena

The **Leibniz-Institute for Natural Product Research and Infection Biology - Hans-Knoell-Institute -** invites applications as

Head of the Department
Microbial Pathogenicity Mechanisms

The Head of the Department will be appointed as a

W3-Professor Microbial Pathogenicity

in a joint recruitment procedure with the **Friedrich-Schiller-University Jena.**

The Leibniz-Institute for Natural Product Research and Infection Biology carries out research on natural products and the infection biology of human-pathogenic fungi with the goals to understand natural products as mediators of biological communication and to isolate novel antifungal drugs. Further information about the mission of the institute is available at **http://www.HKI-Jena.de**

We are interested in investigators with strong research programs in molecular mechanisms of fungal infections, preferentially *Candida albicans*. Candidates should have a postdoctoral lecture qualification (Habilitation) or equivalent degree with a track record of research as evidenced by publications. The successful candidates will be expected to establish a competitive research program and teach Microbial Pathogenicity in master courses of biology and biochemistry.

Excellent knowledge is expected in the fields infection biology, molecular biology, microbiology and biochemistry. Additionally, the candidate should have experiences in the execution of animal experiments.

Qualified female scientists are encouraged to apply for this position. Priority is given to physically handicapped candidates with equal qualifications.

Candidates with an outstanding record of research achievement should send a CV, list of publications with reprints of key papers, and a short statement of research interests and scientific goals until **August 31, 2005** to:

Friedrich-Schiller-Universität Jena
Biologisch-Pharmazeutische Fakultät
- Dekanat -
Fürstengraben 25, 07743 Jena/Germany
Tel.: ++49-(0)3641-949 000, Fax: 949 002

The National Institute of Biological Sciences

The National Institute of Biological Sciences (NIBS), Beijing invites applications to fill multiple Principal Investigators (PIs) positions. The successful candidates are expected to run their own independent research in this modern research institute studying mechanism-based aspects of biological sciences. Candidates who study molecular and biochemical aspects of cell biology, cancer biology, animal model of diseases, metabolism, and chemical biology are particularly welcome to apply.

The candidate should have a Ph.D. degree and several years of postdoctoral training. The initial appointment will be for 5 years, with full support by the NIBS. Renewal of appointment with support will be based on merit and is conditional upon passing reviews by international committees. In addition to wide open modern laboratory space, generous start-up packages, and internationally competitive salaries, NIBS aims to provide PIs with an exciting environment, collegial and interactive colleagues, efficient administrative and support mechanisms, first rate technology core facilities, outstanding graduate students, opportunities for substantial communications with the international scientific community. NIBS is particularly experienced in helping young scientists who are fresh out of their postdoctoral training to establish their laboratories quickly and efficiently so that they can start doing significant and creative research without much delay. The Principal Investigators offered by NIBS should start to do their research in the end of 2005.

Applicants should send in their CVs and research interests (limited to 3 pages) in English to the following email address: **wangtao@nibs.ac.cn** or **wangt71@yahoo.com.cn**.

They should also arrange for 3 recommendation letters to be sent to the same email address.

TENURE TRACK FACULTY POSITIONS

Center of Excellence in Oral and Craniofacial Biology
Louisiana State University Health Sciences Center in New Orleans

The departments of Physiology, Pharmacology, and Cell Biology and Anatomy at the LSU Health Sciences Center in New Orleans are seeking applicants for several 12-month tenure-track faculty positions at the Assistant or Associate Professor level. Successful candidates will participate in the expanding Center of Excellence in Oral and Craniofacial Biology at the LSU School of Dentistry. Applicants should have a Ph.D. and/or D.D.S. or M.D. degree. Candidates should have research interests in **cellular/molecular signaling mechanisms** in any one of a wide variety of foci including:

- Inflammation
- Pain
- Infectious disease
- Cancer

Those working in an oral biology related field are especially encouraged to apply. Our goal is to continue to develop the Center with talented investigators with shared interests. We are seeking individuals with outstanding potential for funding or who have demonstrated the ability to establish independent funding and who are also committed to participate in the educational mission of the Health Sciences Center. Applicants are encouraged to visit our website for details about the Health Sciences Center (**www.lsuhsc.edu**), departments **www.medschool.lsuhsc.edu/cell_biology/**, **www.medschool.lsuhsc.edu/physiology/**, **www.medschool.lsuhsc.edu/pharmacology/**, and dental school (**www.lsusd.lsuhsc.edu**) including the Center of Excellence (**www.lsusd.lsuhsc.edu/research/center_excellence.htm**).

Interested candidates should submit electronically by pdf, a curriculum vitae with publications, statement of present and future research goals, and names and addresses of three references, to the attention of: **Dr. Paul L. Fidel, Director of the Center of Excellence for Oral and Craniofacial Biology, LSU School of Dentistry.** Applications should be sent to **Sarah Rogover** (**srogov@lsuhsc.edu**) by **September 30, 2005**.

LSUHSC is an AA/EOE.

TENURE TRACK POSITION IN ANATOMY AND CELL BIOLOGY

Indiana University School of Medicine – Northwest on the Indiana University Northwest campus in Gary, Indiana is seeking applicants for a tenure-track position in Anatomy and Cell Biology at the Assistant/Associate Professor level. Applicants must have a Ph.D., M.D., or equivalent degree and postdoctoral training is preferred. Demonstrated expertise in teaching medical histology and cell biology (including laboratory) and a willingness to teach in a problem based learning (PBL) curriculum are required.

The ideal candidate will develop and maintain an innovative, externally funded research program that complements ongoing programs in eye research. Current areas of expertise include corneal innervation, corneal wound healing, retinal pigment epithelial cell biology, and ocular epithelial transport processes (refer to **http://shaw.medlib.iupui.edu/nwcme/nwcme.html**). The successful candidate will occupy research space in a new building that opened in 2004.

Qualified applicants should submit a cover letter, curriculum vitae, description of research and teaching experience, and arrange for three letters of reference to be sent by **September 1, 2005** to:

Carl Marfurt, Ph.D., Chair
Search and Screen Committee
IUSM – Northwest
3400 Broadway
Gary, IN 46408

or by e-mail to **cmarfurt@iun.edu**

Indiana University is an EEO/AA Employer, M/F/D.

University of Zurich

The medical faculty of the **University of Zürich** seeks to fill the Position of an

Assistant Professor in Orthopaedic Biomechanics

within the Department of Orthopaedics located at the Balgrist Hospital, Zürich.

We are searching for outstanding individuals with a background in Biomechanics and strong interest in Mechanobiology - in particular in biological mechanism for bone and muscle regeneration on a molecular and cellular level. The successful candidate will have an impressive research record and will be expected to establish an independent research group in the division of Orthopaedic research at the Balgrist Hospital.

The ideal candidate will be expected to attract substantial independent funding in his/her field of research and will be encouraged to take active interest in the complementary clinical and basic research programs of the Department of Orthopaedics.

Interested applicants should send (**in duplicate**) the entire CV, including a list of publications, a record of independent funding and a short summary of the research interest, together with the names of 3 referees to the Dean's Office, Medical Faculty of the University of Zürich, Search Committee Coordination, Zürichbergstrasse 14, CH-8091 Zürich, Switzerland. Deadline for applications is October the 30th 2005.

For further information, please contact the president of the Search Committee, Prof. Dr. med. Peter Groscurth, Institute of Anatomy, University of Zürich, Winterthurerstrasse 190, CH-8057 Zürich, Switzerland (gc@anatom.unizh.ch) or the Chairman of the Department of Orthopedics, Prof. Dr. med. C. Gerber (Christian.gerber@balgrist.ch).

Detailed application instructions can be obtained from the Dean's office, FAX +41 44 634 10 79 or from the Faculty web site: www.med.unizh.ch/FormulareundRichtlinien/Bewerbung.html

The University of Zürich is an equal opportunity employer.

Sponsored by MERCK Research Laboratories Boston

Attend the next *Science* Career Fair

10 August 2005
11:00 a.m.–4:00 p.m.
Boston Convention Center
Room 253
Boston, MA

Science is hosting this career fair in conjunction with **Drug Discovery Technology™ 2005**

Scientists

Scientists at all levels are invited to meet recruiters face to face.

Attendance is free.

Visit ScienceCareers.org and click on Career Fairs for more information and a list of exhibitors.

Experts will be on hand to help you refine your resume.

Post your resume

Exhibitors have access to *Science*'s Resume/CV Database prior to the event, so post your resume today at ScienceCareers.org.

Exhibitors

Meet scientists with varying experience and backgrounds.

Exhibitor rate: $3,000

Free access to *Science*'s Resume/CV Database through 31 August when you sign up to exhibit at the career fair.

To exhibit, contact Daryl Anderson:
Phone ..... (202) 326-6543
Fax ..... (202) 289-6742
E-mail ... advertise@sciencecareers.org

ScienceCareers.org
We know science AAAS

POSITIONS OPEN

HEAD, DEPARTMENT OF PHYSIOLOGICAL SCIENCES
Center for Veterinary Health Sciences
Oklahoma State University

The Department of Physiological Sciences invites applications and nominations for the position of Head, Department of Physiological Sciences, to be filled at the rank of tenured **PROFESSOR** effective January 1, 2006. In addition, the successful candidate will hold an Endowed Professorship in the College.

The Department of Physiological Sciences is part of the Oklahoma State University College of Veterinary Medicine and encompasses the disciplines of physiology, anatomical sciences, pharmacology, and toxicology. The Department has broad research interests in these disciplines as well as a significant commitment to teaching in both the graduate and D.V.M. professional curricula.

The Department Head is the chief administrative officer with responsibility for the instructional programs of the Department; administrative, budgetary, and promotion decisions; and for providing strong leadership in the development of research, teaching, and public service. The position is 40 percent administration and 60 percent research/instruction. Candidates must have an earned Doctorate; achieved national and international recognition for their scholarship appropriate to the rank of Professor; and have a distinguished scholarly record. Desirable qualifications include, but are not limited to, documented leadership skills, previous administrative experience in a doctoral-granting program, a history of external funding, experience with program development in research and education, evidence of strong communication and organizational skills, and evidence of commitment to working with and supporting a diverse student and faculty population. Applicants possessing the credentials for such a position should submit a full resume including a list of publications and the names, addresses, and telephone numbers of at least five references. Salary is commensurate with experience. A starting date of January 1, 2006, is desirable. To ensure full consideration, applications should be received by September 15, 2005. Applications will be accepted until the position has been filled. Interviews may take place prior to the application deadline; however, no final decision will be made until after that date. Send applications and nominations to:

Jerry R. Malayer, Ph.D.
Professor and Associate Dean for Research and Graduate Education
Center for Veterinary Health Sciences
Oklahoma State University
222 McElroy Hall Stillwater, OK 74078
Telephone: 405-744-8085
E-mail: malayer@cvm.okstate.edu

Oklahoma State University is an Affirmative Action/Equal Employment Opportunity Employer, committed to multicultural diversity.

The Cancer Center at the University of Chicago seeks a **TECHNICAL DIRECTOR** of its Human Immunologic Monitoring Facility. This laboratory performs quantitative assays of immune response parameters, along with biochemical and molecular correlates, on samples obtained from patients participating in clinical studies. The analyses are for research purposes only, and therefore new assays are continuously being developed to fit the needs of specific protocols. The position offers opportunities for parallel scientific investigations and independent funding. The laboratory works closely with the institutional current good manufacturing practice facility so that the generation of investigational products and scientific monitoring of patients are well integrated. The candidate should have a Ph.D. in immunology or equivalent, and experience working with human cells. Salary and rank commensurate with experience. Please send curriculum vitae along with a description of relevant experience to: **Thomas F. Gajewski, M.D., Ph.D., University of Chicago, 5841 S. Maryland Avenue, MC2115, Chicago, IL 60637. E-mail: tgajewsk@medicine.bsd.uchicago.edu.** *The University of Chicago is an Affirmative Action/Equal Opportunity Employer.*

POSITIONS OPEN

ASSOCIATE PROFESSOR/PROFESSOR
Neuropharmacology

We seek an established investigator with outstanding research accomplishments in neuroscience and neuropharmacology. Applicants must have expertise and a strong record of publications related to mechanisms of memory and cognition and the effects of pharmacological and/or toxicological agents on these processes. Applicants with expertise in drug discovery and development related to neurological and psychiatric disorders are particularly encouraged to apply. A past and current record of substantial extramural funding including NIH grants is also required. We offer a generous startup package and outstanding facilities are available for electron microscopy, cell imaging, microarray technology, genetically modified animals, primate research, small animal behavior, and clinical collaborations. The successful applicant will participate in teaching programs for professional and graduate students. Prior experience with professional students is desired. Please send curriculum vitae, summary of professional and research goals, and the names and addresses of three references to: **R. William Caldwell, Ph.D., Department of Pharmacology and Toxicology, Medical College of Georgia, Augusta, GA 30912-2300. E-mail: wcaldwel@mail.mcg.edu** and visit the Medical College of Georgia (MCG) homepage (**website: http://www.mcg.edu**). Application review will begin July 29, 2005. Deadline for applications is August 14, 2005. *MCG is an Equal Employment Opportunity/Affirmative Action/Equal Acess Employer.*

NEUROIMAGING FACULTY
Department of Neurosciences
College of Medicine
Medical University of South Carolina, Charleston

The Department of Neurosciences at the Medical University of South Carolina (MUSC) invites applications for a **TENURE-TRACK FACULTY POSITION** in the general area of human brain imaging. This position offers a competitive and generous startup package and provides a unique opportunity to use the basic and clinical imaging resources at MUSC to grow an area of advanced interdisciplinary research based on human brain imaging. The faculty member (**ASSISTANT, ASSOCIATE,** or **FULL PROFESSOR**) will closely collaborate with basic and clinical neuroscientists, other MUSC researchers from the Center for Advanced Imaging Research (CAIR) and imaging researchers at the University of South Carolina (USC) and The University of Nottingham (Nottingham, United Kingdom) through the statewide Brain Imaging Center of Excellence. The position requires an M.D. and/or Ph.D., a record of extramural grant funding in the area of human brain imaging, and a demonstrated ability to work with an interdisciplinary research team. Depending on the candidate's interests and qualifications, the successful candidate may also assume the role of CAIR Director.

Review of applications will begin on September 1, 2005, and continue until the position is filled. Applicants must apply online at **website: http://www.musc.edu/hrm/careers/faculty.htm** (position/requisition number 041747). Your online application for this position should also include curriculum vitae, the names and contact information of at least three references, and a cover letter expressing your qualifications and statement of research interests addressed to: Mark S. George, M.D., Chair, Neuroscience Imaging Search Committee, Department of Neurosciences, Medical University of South Carolina, 173 Ashley Avenue, BSB 403, Charleston, SC 29425.

Nominations of qualified individuals are also welcome. The nominee's curriculum vitae should be sent via e-mail to: **Mark George, M.D., Chair, Neuroscience Imaging Search Committee, c/o Cheri Kubalak** at **e-mail: kubalakc@musc.edu.** *MUSC is an Equal Employment Opportunity/Affirmative Action Employer.*

POSITIONS OPEN

ASSISTANT OR ASSOCIATE PROFESSOR/ ASSISTANT OR ASSOCIATE SCIENTIST
POSITION #38286/80329
Department of Animal Sciences
Washington State University

Assistant or Associate Professor/Assistant or Associate Scientist, Department of Animal Sciences, Washington State University. Assistant or Associate Professor, tenure-track, 12-month faculty position, 85 percent research and 15 percent teaching. Required: (1) A Ph.D. in animal/veterinary Sciences, biological sciences, molecular biology, or related disciplines at time of application; (2) a demonstrated ability to communicate effectively and a strong publication record; (3) postdoctoral experience is required for hiring at the assistant professor level; (4) and an established record of extramural funding is required at the associate professor level. Application: Screening begins October 5, 2005. Questions regarding this position can be directed to: **Dr. Jerry J. Reeves, Search Committee Chair** at **telephone: 509-335-8339** or **e-mail: reevesjj@wsu.edu.** For position description, visit **website: http://www.hrs.wsu.edu/employment/FAPvacancies.asp** (search #4060). Contact: **Jaimie Dahl, Department of Animal Sciences, P.O. Box 646310, Pullman, WA 99164-6310. Telephone: 509-335-5523; fax: 509-335-1082; e-mail: jaimie@wsu.edu.** *Equal Employment Opportunity/Affirmative Action/ADA.*

ENDOWED CHAIR IN CLINICAL IMMUNOLOGY
Tulane University School of Medicine

The Department of Medicine of the Tulane University Medical School invites applications and nominations for the position of the Herbert J. Harvey, Jr. Chair in Clinical Immunology. Candidates should have a strong commitment and track record in basic and clinical research in the area of immunology. They should be board certified in a relevant subspecialty of internal medicine and be eligible for a tenure-track position on the faculty of the Department of Medicine. The Chair carries significant salary support in order to give its holder protected time for a commitment to research. Preference will be given to candidates who currently have strong ongoing externally funded projects.

Please address inquiries and correspondence to:

Blackwell Evans, M.D.
Associate Professor of Medicine
Chair of Harvey Chair Search Committee
Department of Medicine – SL 16
1430 Tulane Avenue
New Orleans, LA 70112

Tulane University School of Medicine is an Equal Opportunity Employer/Affirmative Action Institution. Women and minority candidates are urged to apply.

STANFORD UNIVERSITY
Department of Chemistry

A **FACULTY POSITION** open at any rank is available in the general area of organic chemistry. We especially welcome applicants starting on the tenure track. Appointment will commence on or after September 1, 2006. Completed applications must be received by October 1, 2005. To ensure full consideration of your application, please ensure that all letters of reference arrive by this deadline. Applicants must be strongly motivated toward creative research and committed to teaching at the undergraduate and graduate levels. Applications must include the following materials: (1) current curriculum vitae and list of publications, (2) brief statement of research interests, and (3) three letters of reference sent directly to the search committee, on your behalf.

Applications and supporting materials should be addressed as follows: **Chair, 2005-06 Organic Chemistry Search Committee, Department of Chemistry, Stanford University, Stanford, CA 94305-5080.** *Stanford University is an Equal Opportunity/Affirmative Action Employer.*

POSITIONS OPEN

UCLA

POSTDOCTORAL POSITION available at the University of California at Los Angeles (UCLA) to study mechanisms of signal transduction and gene regulation via the calcium/calmodulin-dependent protein kinase cascade pathway in neurons and lymphocytes. Requires strong background in molecular biology, signal transduction, and tissue culture techniques. Send curriculum vitae and names of three references to: **Dr. Talal Chatila, Department of Pediatrics, UCLA. E-mail: tchatila@mednet.ucla.edu.**

COGNITIVE/BEHAVIORAL NEUROSCIENTIST

The Bates College Department of Psychology and Program in Neuroscience invite applications for a tenure-track position in cognitive/behavioral neuroscience at the rank of **ASSISTANT PROFESSOR,** beginning September 2006. The successful candidate will have a strong commitment to undergraduate teaching (typically four courses per year including laboratories) and supervision of undergraduate research. The candidate should be able to teach introduction to neuroscience, cognitive neuroscience, a course in their specialty, and additional courses in psychology/neuroscience. Candidates should hold a Ph.D. in neuroscience, psychology, or a related field. The Department has nine faculty members. See **website: http://www.bates.edu/acad/depts/psychology/index.html.** Laboratory facilities for animal research are excellent, and human research facilities are located in a new building.

Founded in 1855, Bates is one of the nation's leading liberal arts colleges, with a long history of commitments to principles of human dignity and diversity. Bates has highly competitive admission, graduates over 85 percent of its entering students, and over half of its alumni earn graduate degrees. Bates has 1,700 students, 200 faculty members, and 550 staff and administrative employees. The College is proud of its strong involvements in the Lewiston-Auburn communities, Maine's second largest population center, with a population of approximately 65,000. The College is 40 minutes from Portland and the Maine coast, and two-and-a-half hours north of Boston.

Persons interested in sharing the position are encouraged to apply. Review of applications begins October 15, 2005, and will continue until the position is filled. Please mail letter of application, curriculum vitae, three letters of recommendation, graduate and undergraduate transcripts, statements on teaching and research, and reprints to:

Cognitive/Behavioral Neuroscience Search (#R2469)
c/o Bates College Academic Services
2 Andrews Road, 7 Lane Hall
Lewiston, ME 04240
Website: http://www.bates.edu

Bates College values a diverse college community and seeks to assure Equal Opportunity through a continuing and effective Affirmative Action program.

INORGANIC CHEMISTRY SEARCH

California Institute of Technology invites applications for a tenure-track position as **ASSISTANT PROFESSOR** specializing in inorganic chemistry with an initial appointment of four years, contingent upon completion of all requirements for a Ph.D. in chemistry or other related field. Outstanding candidates with a strong commitment to research and teaching excellence are encouraged to apply. Submit curriculum vitae, publication list, a description of proposed research, and three letters of recommendation to: **Chair of the Inorganic Chemistry Search Committee, M/C 127-72, California Institute of Technology, Pasadena, CA 91125.** Applications should be received by October 15, 2005. *The California Institute of Technology is an Equal Opportunity/Affirmative Action Employer. Women, minorities, veterans, and disabled persons are encouraged to apply.*

POSITIONS OPEN

CHAIR
Department of Chemistry

The University of Nebraska-Lincoln (UNL) invites applications and nominations for Professor and Chair of the Department of Chemistry. We are seeking candidates with an outstanding research program, a history of strong external funding, demonstrable leadership ability, excellent interpersonal skills, and a vision for enhancing the research and educational programs of the Department. Candidates should have a Ph.D. in chemistry or a closely related field. The position may be associated with an endowed professorship.

The Department has a long tradition of excellence in both teaching and research. We are an American Chemical Society-certified program offering both B.S. and B.A. chemistry degrees. Departmental courses also support campus liberal arts requirements as well as the degree programs of a number of other majors. The only Ph.D.-granting chemistry program in the state and one of the premier research units within the University, the Department is housed in Hamilton Hall, an eight-story 200,000 square-foot facility dedicated to research and teaching in chemistry. A complete renovation of nearly half the Department's research space, underwritten with two NIH infrastructure grants, will be completed by early 2006. Research programs span traditional and interdisciplinary areas of chemistry, including materials science, biotechnology, structural biology/proteomics, water science, environmental toxicology, and cancer research. The Department anticipates a number of faculty hires at all levels as part of strategic campus initiatives in several interdisciplinary areas. The Department has strong support from alumni and is assisted by an active Industrial Advisory Board. Additional information about the Department can be found at **website: http://www.chem.unl.edu.**

Nominations and applications should be sent to: **Chemistry Chair Search Committee, College of Arts and Sciences, 1223 Oldfather Hall, University of Nebraska-Lincoln, Lincoln, NE 68588-0312. E-mail: chemsearch@unl.edu.** Applications materials should include a cover letter, curriculum vitae with a full list of publications, a summary of past, current, and pending research support, the names of three references, and a brief statement of research, educational, service, and administrative interests. Review of applications will commence September 30, 2005, and will continue until the position is filled. We assure reasonable accommodation under the Americans with Disabilities Act; contact the search committee (**telephone: 402-472-3634** or **e-mail: chemsearch.unl.edu**) for assistance. *The University of Nebraska is committed to a pluralistic campus community through Affirmative Action/Equal Opportunity and is responsive to the needs of dual career couples.*

NIH-funded **POSTDOCTORAL POSITION** available to study mechanisms underlying ethanol/withdrawal and (estrogen) neuroprotection using animal and cell culture. Experience in protein purification, biochemistry, and/or behavior is preferred. Conducting and designing experiments and writing manuscripts will be required. Salary per year: USD $32,000 to $38,000 plus fringe benefits. E-mail curriculum vitae to: **Dr. Marianna Jung (e-mail: mjung@hsc.unt.edu), Pharmacology/Neuroscience, University of North Texas Health Science Center at Fort Worth, TX 76107.** See publications: *Eur. J. Pharm.* **515:**62, 2005; *Exp. Biol. Med.* **230:**8, 2005; *Neur. Lett.* **377:**44, 2005. *The University of North Texas Health Science Center is an Equal Employment Opportunity/Affirmative Action Institution.*

POSTDOCTORAL POSITION
Yale School of Medicine

A Postdoctoral position is available to study the structure and function of bacterial homologs of Alzheimer's gamma-secretase. Experience in membrane protein biochemistry is required. Please send curriculum vitae, brief description of career goals, and names of three references to: **Dr. Ya Ha, 333 Cedar Street, New Haven, CT 06520, U.S.A. E-mail: ya.ha@yale.edu.**

POSITIONS OPEN

Duke Comprehensive Cancer Center NCI CCC
DUKE UNIVERSITY HEALTH SYSTEM

POSTDOCTORAL POSITIONS IN CANCER IMMUNOTHERAPY
Program in Molecular Therapeutics

The laboratories of **H. Kim Lyerly, M.D., Tim Clay, Ph.D.,** and **Gayathri Devi, Ph.D.** have Postdoctoral positions available for outstanding individuals interested in immunotherapy, gene therapy, immune homeostasis, stem cell biology, vaccines, and signal transduction research. Studies encompass basic and translational research projects using mouse models and human clinical samples. Applicants with a proven track record of productivity (publications and/or Ph.D. thesis) and experience in immunology, cell signaling, and/or molecular biology are encouraged to send their curriculum vitae, a letter of research interests, and contact information for three references to:

Tim Clay, Ph.D.
Duke University Medical Center
Medical Sciences Research Building, Room 401
Box 2606
Durham, NC 27710
E-mail: tim.clay@duke.edu

BIOCHEMIST/MOLECULAR BIOLOGIST
Bryn Mawr College

The Department of Biology invites applications for a tenure-track faculty position in biochemistry/molecular biology at the rank of **ASSISTANT PROFESSOR.** We are searching for an individual who will thrive in an environment that combines teaching and research. The successful candidate is expected to teach at all levels of the curriculum and establish an externally funded research program that provides rigorous collaborative research projects for undergraduates. Candidates with research interests that complement the College's interdisciplinary program in environmental studies are particularly encouraged to apply. A Doctorate and at least one year of postdoctoral research experience are required. Submit curriculum vitae, statement of research and teaching interests, and arrange for three letters of recommendation to be sent by October 3, 2005, to: **Chair, Biology Search, Department of Biology, Bryn Mawr College, and 101 N. Merion Avenue, Bryn Mawr, PA 19010-2899.** Located in suburban Philadelphia, Bryn Mawr College is a highly selective liberal arts college for women who share an intense intellectual commitment, a self-directed and purposeful vision of their lives, and a desire to make meaningful contributions to the world. Bryn Mawr comprises an undergraduate college with 1,200 students, as well as coeducational graduate schools in some humanities, sciences, and social work. The College supports faculty excellence in both teaching and research, and participates in consortial programs with the University of Pennsylvania, and Haverford and Swarthmore Colleges. *Bryn Mawr College is an Equal Opportunity/Affirmative Action Employer. Minority candidates and women are especially encouraged to apply.*

POSTDOCTORAL/RESEARCH ASSOCIATE

Position available immediately to work on project to study regulation and function of human cystathionine beta-synthase and homocysteine related disease (see: *Circ. Res.* **94:**1318–24, 2004; and *J. Biol. Chem.* **279:**52082–6, 2004). Preferable experience includes protein biochemistry, enzymology, molecular biology, yeast genetics, and mouse genetics. Successful candidate should be highly motivated and capable of independent research. Acceptance for this position will require an interview in Philadelphia. Travel funds are available for qualified individuals traveling within North America. Send curriculum vitae and names of three references to: **Dr. Warren Kruger, Division of Population Science, Fox Chase Cancer Center, 333 Cottman Avenue, Philadelphia, PA 19111. E-mail: wd_kruger@fccc.edu.** *Equal Opportunity Employer.*

Career advice, insight and tools.

Turn to the experts for the big picture.
Visit www.ScienceCcareers.org

Your career is too important to leave to chance. So to find the right job or get career advice, turn to the experts. At ScienceCareers.org we know science. And we are committed to helping take your career forward. Our knowledge is firmly founded on the expertise of *Science*, the premier scientific journal, and the long experience of AAAS in advancing science around the world. Put yourself in the picture with the experts in science. Visit www.ScienceCareers.org.

ScienceCareers.org
We know science AAAS

POSITIONS OPEN

POSTDOCTORAL, RESEARCH, AND CLINICAL FELLOWSHIPS
at the
National Institutes of Health
U.S. Department of Health and Human Services

Website: http://www.training.nih.gov

NIH is dedicated to building a diverse community in its training and employment programs.

ASSOCIATE RESEARCH SCIENTIST

A position is available in the Department of Pharmacology at Columbia University College of Physicians and Surgeons for a nontenured research faculty position as Associate Research Scientist. Applicants should possess an M.D. or Ph.D. degree with a background in research on gap junction molecular biology and physiology as evidenced by publications in outstanding journals. The successful candidate must have expertise in a wide range of methods, including molecular biology methodology, the manufacture and use of antibody arrays, surface plasmon resonance, immunofluorescence confocal microscopy, immunoelectronmicroscopy, and laser capture microscopy. He/she must be able to apply these methodologies to the investigation of cardiac gap junctional connections. Candidates must be competitive for extramural funding from the NIH and American Heart Association to support the research program. Salary will be commensurate with prior experience and potential for success within the guidelines of Columbia University for this position. For consideration for this position, interested candidates should submit their curriculum vitae, a statement of research accomplishments, and the names of three references to: **Dr. Andrew L. Wit, Department of Pharmacology, Columbia University College of Physicians and Surgeons, 630 W. 168th Street, New York, NY 10032.** Electronic applications may be sent to **e-mail: alw4@columbia.edu.** *Columbia University is an Affirmative Action/Equal Opportunity Employer and specifically invites applications from women and minorities.*

ORGANIC CHEMISTRY SEARCH

California Institute of Technology invites applications for a **TENURE-TRACK FACULTY POSITION** in organic chemistry in the Division of Chemistry and Chemical Engineering. Initial appointment at the **ASSISTANT PROFESSOR** level will be for four years with consideration given to exceptionally well-qualified applicants at the **ASSOCIATE** and **FULL PROFESSOR** levels. Appointment will be contingent upon completion of all requirements for a Ph.D. in chemistry or a closely related field. Outstanding candidates who have strong commitments to research and teaching are encouraged to apply. Submit by October 15, 2005, curriculum vitae, a publication list, a concise description of proposed research, and three letters of recommendation to: **Robert H. Grubbs, Organic Faculty Search Committee, MC 164-30, California Institute of Technology, Pasadena, CA 91125.** *The California Institute of Technology is an Equal Opportunity/Affirmative Action Employer. Women, minorities, veterans, and disabled persons are encouraged to apply.*

Positions are available for **POSTDOCTORAL FELLOWS/GRADUATE STUDENTS** in molecular virology, in the area of the role of host proteins in virus replication and recombination. The principal investigator's laboratory developed yeast as an experimental system to study virus replication (*Proc. Natl. Acad. Sci.* **102:**7326–7331) and recombination (*Proc. Natl. Acad. Sci.* **102:**10545–10555). Applicants with a background in molecular biology or biochemistry are encouraged to apply to: **Dr. Peter Nagy, University of Kentucky, Department of Plant Pathology, 201F Plant Science Building, 1405 Veterans Drive, Lexington, KY 40546-0312. Telephone: 859-257-7445, extension 80726. Fax: 859-323-1961. E-mail: pdnagy2@uky.edu.**

TENURE-TRACK FACULTY POSITIONS IN PROTEOMICS/BIOLOGICAL MASS SPECTROMETRY
Aab Institute of Biomedical Sciences
University of Rochester Medical Center

Two tenure-track positions are available at the **ASSISTANT** or **ASSOCIATE PROFESSOR** level for candidates with research specialization in biological applications of mass spectrometry. Areas of potential focus might include proteomics, metabonomics, instrumentation development, or other topics leading to the establishment of an independent, competitive research program that is extramurally funded. The successful candidates are expected to participate actively in graduate training and will have modest teaching requirements. The Medical Center offers exciting opportunities for interdisciplinary and translational research programs with an interactive environment and strong programs in cancer and stem cell biology, craniofacial and oral biology, hematopoiesis, genetics in model organisms, genomics, proteomics, and biomathematics. Candidates should have a strong record of accomplishment. Appointments will be through the Department of Pediatrics or the Center for Oral Biology, the Wilmot Cancer Center, and the Department of Biomedical Genetics.

To apply, submit curriculum vitae, a statement of research interests/plans and names of three references to: **Hartmut Land, Chair, Mass Spectrometry Search Committee, Department of Biomedical Genetics** via **e-mail: cancerbio@urmc.rochester.edu.**

Websites: http://www.urmc.rochester.edu/Aab/bg/ and **http://www.urmc.rochester.edu/GEBS/ggd/.**

The University of Rochester is an Equal Opportunity/Affirmative Action Employer.

POSTDOCTORAL POSITIONS
Protein Synthesis and Synthetic Biology
Vanderbilt University

Join the Pharmacology Department ranked no. 1 nationally in citations per faculty: **website: http://www.vanderbilt.edu/pharmacology.** For details, see laboratory **website: https://medschool.mc.vanderbilt.edu/forster.** Minimal qualifications include expertise in molecular biology and two first-authored research papers in international peer-reviewed journals. Experience with translation or RNA is a plus. The University is located on a single, beautiful campus in the livable "Music City." Please mail a letter of interest, curriculum vitae, and names, e-mail addresses, telephone numbers of three references to: **Anthony C. Forster, M.D., Ph.D., Department Pharmacology and Vanderbilt Institute of Chemical Biology, PRB 459, Vanderbilt University Medical Center, 23rd Avenue South at Pierce, Nashville, TN 37232, U.S.A.** *Vanderbilt University is an Affirmative Action/Equal Opportunity Employer. Women and minority candidates are encouraged to apply.*

RESEARCH SPECIALIST. Project team leader in pollen allergy research, laboratory of **Dr. Daphne Preuss,** The University of Chicago, Chicago, Illinois. Requires Ph.D. plus postdoctoral experience. Expertise in animal cell culture, protein, and small molecule purification, and molecular biology strongly desired. Must have excellent written and verbal communication skills. Position includes supervision of small research team, grant and manuscript preparation, and an individual research project. Send resume to **e-mail: c-breshock@uchicago.edu.**

POSTDOCTORAL POSITION

Study molecular mechanisms of neuronal cell death with emphasis on strategies to overcome stress-induced suppression of protein synthesis. Expertise in cell cultures, molecular biology, and biochemistry desired. Send curriculum vitae and three references to: **W. Paschen, Ph.D., Duke University Medical Center, Department of Anesthesiology, Box 3094, Durham, NC 27710. E-mail: wulf.paschen@duke.edu.**

UNIVERSITY OF HOUSTON
Learning. Leading.

POSTDOCTORAL POSITION

A full-time, Postdoctoral position is available in the Department of Pharmacological and Pharmaceutical Sciences, University of Houston College of Pharmacy. The successful candidate will study the mechanisms of neurodegeneration in a chronic mouse model of Parkinson's disease and to examine the impact of exercise as a neuroprotective approach. Qualified applicants should have a Ph.D. or equivalent degree in pharmacology, neuroscience, or related medical science disciplines. Previous experience in brain research, cell culture, high performance liquid chromatography, animal handling and treatment, immunohistochemistry, in situ hybridization, and other molecular biology techniques are desirable. The position is available immediately. Applicants should send a cover letter, resume, samples of publication, and the contact information of three references to:

Dr. Vincent Lau
Department of Pharmacological and Pharmaceutical Sciences
University of Houston
College of Pharmacy
521 Sciences and Research II
Houston, TX 77204-5037

The University of Houston is an Affirmative Action/Equal Opportunity Employer. Minorities, women, veterans, and persons with disabilities are encouraged to apply.

POSTDOCTORAL FELLOWSHIPS
Colorado State University, Fort Collins

We are seeking person(s) with research experience in the areas of virology/molecular biology/stem cell biology/immunology to participate in NIH and Pediatric Dengue Vaccine Initiative (PDVI) funded projects on HIV/AIDS gene therapy and Dengue viral pathogenesis. Work involves contemporary and emerging technologies in human embryonic stem cells, hematopoietic stem cells, viral vectors, ribozymes, short interfering RNAs (RNAi) and SCID-hu mouse animal model. (Representative work: **Banerjea et al,** *Mol. Therapy* **8:**62–71, 2003; **Anderson et al,** *AIDS Research and Therapy* **2:**1–12, 2005). Capacity for independent work and ability to interact productively with colleagues are important.

Applications will be accepted until August 19, 2005, or until suitable candidates are identified; positions are available immediately. Please send a letter of application together with a list of three references to:

Ramesh Akkina, D.V.M., Ph.D., Department of Microbiology, Immunology, and Pathology, 1619, Campus Delivery, Colorado State University, Fort Collins, CO 80523-1619. Telephone: 970-491-1009; e-mail: akkina@colostate.edu.

Colorado State University is an Equal Opportunity Employer.

MOLECULAR NEUROBIOLOGIST

With expertise in regeneration research and stem cell technology. A background in developmental neurobiology is a plus. Research to be conducted in collaboration with clinical neuroscientists. Mentoring residents in neurological surgery in research techniques is expected. Competitive salary and benefits. Please submit curriculum vitae and letters of interest to: **Setti Rengachary, M.D., Department of Neurological Surgery, Wayne State University, 4160 John R, Suite 930, Detroit, MI 48201. E-mail: srengachary@med.wayne.edu.**

Additional job postings not featured in this issue can be viewed online at **website: http://www.sciencecareers.org.** New jobs are added daily!

Manage your job search more effectively by creating an account at **website: http://www.sciencecareers.org.** You can post your resume (open or confidentially) in our database and use it to apply to multiple jobs simultaneously. Track the jobs you have applied to in special tracking folders. Plus, you can create Job Alerts that will e-mail you notification of jobs that match your search criteria.

SYMPOSIA

BioJapan 2005
World Business Forum

Focusing on the Application of Biotechnology for Business

A Networking Opportunity for Biotech People from Worldwide

September 7 [Wed.] ▸ 9 [Fri.], 2005
10:00-17:00
Pacifico Yokohama, Yokohama City, Japan

Organizers **BioJapan 2005 Organizing Committee**
Japan Bioindustry Association (JBA) Japan Health Sciences Foundation (JHSF)
Society for Techno-innovation of Agriculture, Forestry and Fisheries(STAFF)
Japan Biological Informatics Consortium (JBiC) Japan Association of Bioindustries Executives (JABEX)
Japan Pharmaceutical Manufacturers Association (JPMA)
NPO Kinki Bio-industry Development Organization (KBDO)
Nikkei Business Publications, Inc.

Special Support **City of Yokohama** **Kanagawa Prefecture**

Please access to register: **http://expo.nikkeibp.co.jp/biojapan/2005/eng/**

Exhibition hall admission fee becomes free with on-line registration.

GRANTS

Providing Support for U.S. and Canadian Scientists at the Assistant Professor Level

2006 Investigators in Pathogenesis of Infectious Disease

Awards for research that illuminate the interactions between pathogens and human hosts

Deadline: November 1, 2005

$400,000 award over five years for researchers with M.D., D.V.M., or Ph.D. degrees

- Candidates must have an established record of independent research and hold a tenure-track position as an assistant professor or equivalent at a degree-granting institution.
- Genetic, biochemical, immunological, and other approaches are all appropriate.
- Proposals can include cell-pathogen interactions, host-pathogen interactions, novel routes to disease causation in humans, as well as excellent animal models.
- Research on under-studied pathogens, including fungi, metazoan and protozoan parasites, and emerging viruses, is especially of interest.
- Degree-granting institutions in the United States and Canada may nominate up to two candidates, three if at least one of the nominees is a veterinarian.
- Complete program information, eligibility guidelines, and online submission instructions are available on BWF's website at www.bwfund.org
- BWF supports diversity within the scientific community and encourages applications from women and under-represented minority groups.

BURROUGHS WELLCOME FUND

t 919.991.5100
f 919.991.5160
www.bwfund.org
Post Office Box 13901
21 T. W. Alexander Drive
Research Triangle Park, NC 27709-3901

The Burroughs Wellcome Fund is an independent private foundation dedicated to advancing the biomedical sciences by supporting research and other scientific and educational activities.

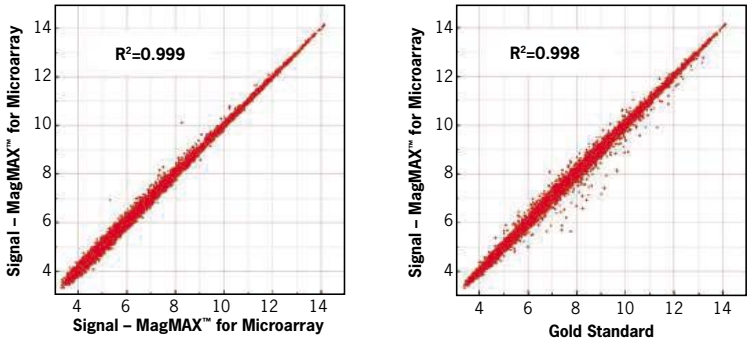
Small Samples. Big Results. A Better Choice.

Ambion's MagMAX™ kits deliver high quality RNA to maximize the success of your gene expression studies.

Advantages of MagMAX technology over glass filter methods:

- Rapid, high throughput isolation of total nucleic acid without cumbersome preprocessing steps
- Higher RNA yields due to more efficient binding and elution of the RNA
- No troublesome filter clogging
- More consistent RNA yields across a variety of sample types and from experiment to experiment
- Low elution volume concentrates RNA for convenient streamlining of downstream applications

Generate the Same High Quality Microarray Data as the "Gold Standard"



Scatter plot of normalized signal intensities of two mouse kidney total RNA samples using MagMAX™-96 for Microarrays and the Gold Standard. The correlation between samples is 0.999. The correlation between the two methods is 0.998. Greater than 60% percent present calls were obtained for each method.

MagMAX™

Magnetic Bead-Based Nucleic Acid Isolation Kits

NEW MagMAX™-96 Blood RNA Isolation Kit

NEW MagMAX™-96 for Microarrays Total RNA Isolation Kit

MagMAX™-96 Total RNA Isolation Kit

MagMAX™-96 Viral RNA Isolation Kit

Learn more at www.ambion.com/prod/magmax

Ambion®
THE RNA COMPANY®

Ambion, Inc.
U.S. 800-888-8804 • Canada 800-445-1161
fax +1-512-651-0201

Ambion (Europe) Ltd
tel +44 (0)1480-373-020
fax +44 (0)1480-373-010

Ambion K.K. (Japan)
tel +81 (0)3-5638-2181
fax +81 (0)3-5638-2182

For a list of Ambion direct free phone numbers and distributors in your country, go to www.ambion.com/contact